ANNUAL REVIEW OF ENTOMOLOGY

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VOLUME 2

PREFACE

The reception accorded Volume 1 of the *Annual Review of Entomology* has been one that holds considerable satisfaction on the part of the Editorial Committee and the Editors. Nevertheless, in so far as it has succeeded, this success is primarily to the credit of the authors who contributed their time and talent, to entomologists generally who have purchased and used the volume, and to the Entomological Society of America which, through its members and officers, has given the effort its enthusiastic support. The favorable response of most authorities who have been invited to contribute reviews in their specialties has been most gratifying, and augurs well for future volumes. These are usually busy persons, but the deadline for receipt of manuscripts is set far enough in advance that the obligations entailed should not be unduly burdensome.

We have especially appreciated the constructive criticisms and suggestions that have been offered by some of the readers, and we hope that the present and succeeding volumes will reflect the benefits of these suggestions. Unfortunately, certain ideals cannot be practically realized because of limitations of space and costs. We should very much like to be able to have a larger volume, use a larger sized type face, include titles of articles cited in the references, and require alphabetized bibliographies only. In each of these instances, however, there are overriding considerations that dictate the procedure followed. In any case, we assure the readers of the *Review* that in so far as it is possible we shall continuously strive to maintain the standards that entomologists in general expect in their publications.

Readers of this volume may have noticed that it contains slightly fewer pages than did Volume 1. Depending largely upon authors being able to meet the deadlines involved, the sizes of the separate volumes may vary somewhat from year to year. Over any three-year period, however, the number of pages will average about 450 pages per volume. This is in accordance with the page limitations presently assigned us by the publisher. Therefore, fewer pages in one volume will mean more pages in another, and vice versa.

Of paramount importance in aiding us to present a volume of first-rate quality, is the support of Annual Reviews, Inc., which we wish again to acknowledge, and especially are we grateful to Mrs. Lillian Rutherford and Mrs. Adele Fumino for their painstaking editorial assistance. Our thanks go also to the George Banta Company, Inc. for its efficient production of the volume.

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TOPICS AND AUTHORS
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DIGESTION IN INSECTS[1,2]

By D. F. Waterhouse

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This review covers a number of the more important aspects of digestive physiology in which there have been significant advances since the relevant chapters in Roeder's Insect Physiology (102) were prepared. Limitation of space has, however, prevented reference to some hundreds of papers which pertain to this chapter.

Peritrophic Membrane

The peritrophic membrane (PM), a single or multi-layered, chitin-containing sheath enclosing the food in the midgut of many insects, is produced either by a ring of allegedly ectodermal cells situated at the anterior end of the midgut (in many Diptera), by the periodic delamination of a layer of material from the general midgut epithelium (in the majority of insects), or by both methods (in Dermaptera and many Lepidoptera). Its main function is said to be to protect the midgut epithelium from mechanical damage, thereby performing the function of the mucus of mammals which is absent from the lumen of the insect midgut. A PM is generally stated to be lacking in most fluid-feeding insects. Much confusion and many contradictory reports about the presence or absence of a peritrophic membrane and the method of its formation have arisen firstly from the lack, hitherto, of suitable microchemical or structural criteria of its presence and secondly, from the widely-held belief that the midgut epithelium, unlike that of the fore- and hindgut, is unable to secrete chitin.

By means of a microadaptation of the van Wisselingh test for chitin, Waterhouse (123) has demonstrated the presence of chitin-containing membranes in many insects where a PM was generally believed to be absent. Amongst fluid-feeding insects it is often present, for example in adult Lepidoptera, in adult Nematocera and Orthorrhapha (Diptera), and has been reported in Corixidae (Hemiptera) [Sutton (109)]. It is clear that many insects which have no need to protect their midgut epithelium from mechanical damage possess a PM.

Waterhouse (123) has also shown that membranes containing chitin are produced by the general midgut epithelium at least in some insects. For example, mosquitoes and many other blood-sucking Diptera [Feng (33); Lewis (64)] form a chitinous membrane around the blood meal in the enlarged posterior midgut. If a second meal is taken before the first is digested, the second meal surrounds the first and distinct membranes can be dis-

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[1] The survey of the literature pertaining to this review was completed in April, 1956.

[2] The following abbreviation is used in this review: PM for peritrophic membrane.

tinguished around both [Waterhouse (123); Yaguzhinskaya (140)]. The anterior midgut is of much smaller diameter than the posterior midgut, so that the chitinous membranes must have been formed *in situ* in the latter region either by delamination from the cell surface or by secretion of a fluid precursor which condenses around the food.

In view of the fact that a PM is possessed by many primitive insects and by representatives of the Onycophora, Crustacea, and Myriapoda [Forster (39); Mason & Gilbert (74); Waterhouse (123)] it is probable that the ancestral insect midgut epithelium was able to secrete chitinous membranes enveloping the food. This capacity has apparently been lost by some insects, but in others it has been restricted to a particular zone of the midgut. It is possible that the well-defined, tubular membranes are the result of the restriction of this capacity to cells at the extreme anterior end of the midgut. The embryonic origin of these cells now requires reexamination for it would be surprising if the loss by the endodermal midgut of the capacity to secrete a PM were so frequently accompanied by the assumption of this function by cells derived from the ectodermal foregut, particularly where one type occurs in the larva and the other type in the adult of the same species (e.g., mosquitoes).

This hypothesis is strongly supported by the work of Rizki (101) who showed that, in *Drosophila* larvae, the PM is secreted by a ring of cells, four cells wide, located in the anterior region of the proventriculus. This ring is posterior to the imaginal cells which separate the fore- and midgut. The ring of cells produces a granular precursor of the PM which becomes homogeneous and plastic on discharge into the gut lumen and is later moulded and condensed into a membrane. The PM in the proventriculus failed to give reactions for protein and lipoid although in other regions of the gut it reacted positively, suggesting contamination subsequent to formation.

Valuable information on the fine structure of the peritrophic membrane has been obtained with the electron microscope. In the majority of insects examined—Odonata, dragonfly nymphs [Edwards & Santos (31)]; Orthoptera, *Periplaneta* [Huber (51); Mercer & Day (77)], *Carausius* [Huber & Haasser (53); Huber (51)], *Locusta* (77); Dermaptera, *Labidura* (126); Coleoptera, *Geotrupes* [Huber (52)], *Melolontha* [Wildbolz (135)]; Lepidoptera, *Tineola* [Lagermalm *et al.* (61)], *Galleria* (77)—the most characteristic and resistant component is a fibrillar network. This is generally formed by three systems of parallel fibrillar strands placed at 60° to each other, producing hexagonal symmetry, but alternatively may consist of two sets of strands at approximately right angles or of various intermediate arrangements. In *Periplaneta* the diameter of the fine fibrils composing the strands is about 100 Å, and there may be several to each strand. The separate strands of a set are about 0.15 to 0.2μ apart [Mercer & Day (77)]. A second component is an amorphous film which fills the interstices and perhaps overlies the network.

These network membranes are produced from the surface of cells carry-

ing a striated border. The suggestion is an attractive one that the "holes" in the network correspond with the spaces occupied by the individual rodlets of the striated border and that fibril formation and orientation occur within the striated border. During detachment of sheets of peritrophic membrane from the border, additional material may be added to produce an overlying and impregnating amorphous layer.

The PM of some insects [*Bombyx*, Huber (51); a noctuid larva, Martignoni (73); *Glossina*, Huber (52); and an adult tabanid, Waterhouse (123)] apparently does not contain a network. Instead, it is composed of many disoriented fibrils lying in an amorphous matrix. In these insects the fluid precursor of the membrane may perhaps be secreted on to the surface of the food so that fibrillation occurs without the orienting influence of the striated border.

The fine structure of the tubular PM formed by a ring of cells at the anterior end of the midgut is still unknown, the unaltered membrane [e.g., from an adult blowfly (Richards & Korda (100)] being too dense or thick for the electron beam to penetrate. However, treatment with pepsin or alkali results in the formation of an intact membrane with reticulations separating thinner areas. An organised fibrillar network can scarcely be expected if the membrane precursor streams continuously off the bottom of the ring of cells producing it. However, from the point of view of the homology of the membranes produced by both methods it will be most interesting to learn whether or not fibril formation occurs in these anteriorly produced membranes. The isolation of networks from the PM of the earwig which produces a tubular membrane anteriorly is taken as proof that the general midgut epithelium also contributes layers to the PM in this insect [Waterhouse (126)].

In addition to chitin it is now clear that protein may enter into the formation of the PM, since protein solvents or proteinases remove the amorphous film from the network membrane [Wildbolz (135)] and produce thinner areas in the blowfly membrane [Richards & Korda (100)].

Whatever the method of production, the PM appears to be present continuously in most insects which produce it, although in some (e.g., adult mosquitoes) it has only been detected after feeding [Waterhouse (123)]. The general midgut epithelium evidently produces individual lamellae intermittently, periods of secretion alternating with periods when the epithelial cells are elaborating further precursor materials. While feeding, zygopterous nymphs form a layer of PM every hour or two. Every 8 hr. or so multilayered membranes are discharged with food residues. Each membrane is secreted by the entire midgut epithelium and varies in length according to the degree of distention of the midgut by food. By contrast, figures now available [Waterhouse (124)] support the conclusion that the anteriorly formed PM is produced continuously and at a rate which is higher in actively feeding than in starving insects. At 30°C. adult earwigs discharge their PM at about 1.6 mm./hr. and food moves through the gut at about the same rate. *Eristalis*

and blowfly larvae produce their continuous tubular peritrophic membranes at a rate of about 5 to 10 mm./hr., whereas food passes through the alimentary canal at a rate between 50 and 75 mm./hr. Perhaps the PM does provide some protection for the epithelium when the rate of food passage is high and particularly in the larvae of many Diptera in which the epithelium is not replaced during the entire larval life. The more rapid passage of food than membrane results in some food particles being left behind the main mass moving within the PM. The continuously produced PM thus ensures that all food residues are passed down the digestive tract at a rate not less than that at which it is formed. In the absence of a PM it is probable that some residue would remain for relatively long periods near the surface of the midgut cells. This happens to materials which have passed out through the PM as shown by the 10 to 20 hr. period during which radio barium can be detected in this situation in *Drosophila* larvae [Bowen (10)]. The fact that the fluid in the space between the PM and the epithelium (containing as it does the products of digestion) moves slowly in relation to the rapidly moving contents of the PM must greatly increase the efficiency of absorption. Furthermore, the presence of a PM must also facilitate the forward movement of fluid in the digestive tract in those species in which this is important. Thus in *Aedes* larvae portion of the fluid from the malpighian tubules passes forwards through the midgut to the anteriorly placed caeca and is there reabsorbed into the haemolymph [Ramsay (97, 99); Wigglesworth (133)].

There is little recent work on the permeability of the PM although Schildmacher (106) has shown that colloidal gold particles of 2 to 4 mμ diameter diffuse through the tubular, anteriorly-produced PM of mosquito larvae, whereas particles of 20 mμ diameter are retained.

ACTION OF BEE PROVENTRICULUS

In the honey bee both honey and pollen are taken into the crop (honey stomach) whose muscles maintain the contents in constant motion. The mechanism whereby pollen grains are removed from the honey has been the subject of much discussion but has now been satisfactorily resolved by Bailey (3). Pollen grains accumulate rapidly in four pouches at the anterior end of the proventriculus which protrudes anteriorly into the crop. The lips of these pouches first separate, filling with nectar, and then collapse together, the nectar emptying back into the crop and the pollen being retained by a fringe of hairs on the edge of the lips. At intervals the pollen is passed on as a bolus into the midgut where it moves down rapidly within the peritrophic membrane to the posterior end. Nectar is also passed on occasionally to the midgut. The mechanism is capable of removing particles varying in size from 3 to 50μ, small particles being filtered more efficiently than larger ones of the same concentration although there is no discrimination between particles of different sizes contained in the same suspension.

A complex proventriculus occurs fairly generally throughout the Hymenoptera. Its primitive function appears to be that of a valve allowing the

insect to use its crop as an efficient storage organ, so that liquid food can be passed on to the midgut as required or regurgitated, as in many social forms. Among the ants it is most elaborately developed in those groups which have the highest form of social behaviour and hence rely most on trophallaxis [Eisner & Wilson (32)]. The development of the filtering ability is probably secondary, but is of considerable value when the insect must digest protein, for there will then be the minimum dilution and inhibition of proteolytic enzymes by sugar solutions. Only species closely related to the honey bee show the same highly efficient adaptation for filtering out pollen [Bailey (4)].

HISTOCHEMISTRY OF THE INSECT GUT

Although there is a wealth of histological information on the gut epithelium, surprisingly little is known about the functions of the cells within the various major regions of the gut. In some insects specialisation of various zones within the mid- and hindgut is suggested by differences in the general appearance of the constituent cells (e.g., the rectal pads) and by variation in the pH or oxidation reduction potential of the digestive juices. Much evidence is now available that, in dipterous and lepidopterous larvae at least [Poulson (88, 89); Poulson & Bowen (90, 91); Poulson et al. (92); Waterhouse (115, 116, 120, 125); Waterhouse & Stay (128); Wigglesworth (134)], there is a far more striking functional differentiation than is indicated by histological studies. Thus, on the basis of the functional attributes of the cells themselves, Waterhouse & Stay (128) have shown that the midgut of blowfly larvae may be divided into anterior, mid, and posterior regions and the mid midgut into five distinct zones. The most interesting and unusual of these is zone II which is a mosaic of two cell types. One type is packed with lipoid spheres and glycogen, contains a cytoplasmic acid phosphatase, and has a distinct striated border. The other cell type accumulates copper, iron (when dietary copper is high), and possesses esterases, cytochrome oxidase, and active dehydrogenases. Glycogen and lipoid are absent, acid phosphatase is weak, and there is no distinct striated border. Poulson & Bowen (91) have shown that many species of *Drosophila* also accumulate iron and copper in specific cells in the larval mid midgut, and other histochemical tests indicate that a mosaic of cells of differing function occurs in this region (126).

The occurrence of two principal cell types (columnar and goblet) in the midgut epithelium of lepidopterous larvae has been known for many years. The columnar cells are similar to the simple epithelial cells that occur in the midgut of many insects. The goblet cells, which appear to be restricted to Lepidoptera, are highly differentiated and possess an internal cavity bordered by a faintly striated lining. Unlike the goblet cells of mammals, those of insects do not contain mucoid material [Day (17)] nor does there appear to be an opening from the goblet cavity into the gut lumen [Waterhouse (119)], materials which move out of the cavity passing through a bounding membrane. Ascorbic acid and alkaline phosphatase are lacking in

the *Tineola* goblet cells, although both are present in the adjacent columnar cells [Day (18, 19)]. However, in *Tineola* and several other lepidopterous larvae, goblet cells play an important part in metal and dye metabolism, high concentrations of both being accumulated in the goblet cavity or in the cytoplasm of the cell. Oxidation-reduction and pH indicators are also accumulated [Waterhouse (120)]. On the other hand, with few exceptions, metal accumulations cannot be detected in the columnar cells [Waterhouse (118)]. One of the functions of the goblet cells appears to be that of storage excretion.

Histochemical tests indicate that the hindgut epithelium of blowfly larvae is an active tissue which plays an important role both in metabolism and in regulating the products of excretion. The hindgut is divided into short anterior and posterior regions (which function principally as sphincters) and a long central region. The central hindgut is most unusual in that it is composed of three cell types, each forming a longitudinal band. The bands produced by cell types A and B each form about one half of the hindgut, whereas the cells of type C form two narrow longitudinal strips one cell wide, situated at the junctions of the bands of A and B cells. The A cells are rich in potassium, acid phosphatase, dehydrogenases, and acetyl esterase. On the other hand, the B cells react strongly for ammonia and stain diffusely for barium in larvae fed on a barium-enriched diet. The C cells react very strongly for ammonia and accumulate barium-rich granules. The central hindgut rapidly takes up ammonia (liberated in quantity by deaminase action) from the haemolymph and the B and C cells are particularly active in this process. Much of the ammonia is eliminated together with bicarbonate.

Ramsay (97) has shown that, in *Aedes detritus* Haliday, but not in *Aedes aegypti* Linnaeus, the anterior part of the rectum is lined with an epithelium distinctly different from that in the remainder of the rectum. This was tentatively correlated with the ability of *A. detritus*, not possessed by *A. aegypti*, to produce a hypertonic fluid in the rectum when kept in saline media. Both species can, however, excrete a fluid hypotonic to the haemolymph when kept in fresh water. Most or all of this fluid is derived from the malpighian tubules, with little or no contribution from the midgut in which the fluid is always iso- or hypertonic [Ramsay (98)].

DIGESTION OF WOOL

The well known resistance of wool to attack by proteolytic enzymes and its relative insolubility in the usual protein solvents is considered to be attributable both to its chemical and its morphological structure [Geiger & Harris (40)]. The wool protein, keratin, also occurs in hair, feathers, and other epidermal structures of vertebrates. It is characterized by a high, but varying content of cystine, the sulphur of which forms disulphide bonds between adjacent polypeptide chains. These disulphide bridges contribute greatly to the stability of the keratin molecule and, when they are destroyed, the protein becomes more soluble and more readily digested.

So far as is known the only animals able to digest keratin are all insects, namely the bird-infesting Mallophaga or chewing lice, the larvae of some dermestid beetles, and the larvae of a few moths belonging, or related, to the family Tineidae [Ott (86); Pradhan (96); Waterhouse (121, 122)]. These insects evidently must possess some unusual digestive mechanism enabling them to degrade such a widespread and abundant material as keratin, which is nutritively useless to other animals. Many factors probably contribute to this mechanism, and among those factors which appear to play a relatively minor part we may list mechanical damage [Day (20)] and alkaline digestive juices (in the clothes moth about pH 10), which would tend to render the disulphide links less stable. However, alkalinity is typical of lepidopterous larvae rather than of wool-digesting insects [Waterhouse (117, 120, 121, 122)]. Furthermore, no differences of significance in wool digestion were found when proteinases from *Tineola* larvae and other insects are compared with vertebrate trypsin, using pH optima and effect of activators, inhibitors, and redox potential as criteria [Powning, Day & Irzykiewicz (95)]. Bacteria play a negligible role in keratin breakdown in clothes moth larvae [Crewther & McQuade (16)].

It is quite clear now that, as suggested many years ago by Linderstrøm-Lang & Duspiva (66), one essential difference between *Tineola* larvae and other insects unable to digest wool is the unusually reducing conditions (-200 to -250 mv.) which are maintained in the larval midgut [Waterhouse (120, 121, 122)]. Under these conditions reduction of disulphide bonds to sulphydryl groups occurs (a cystine moiety becoming two of cysteine). The reduced keratin so formed can be digested quite readily by proteases from *Tineola* and many other sources. Some attention has been paid to the factors responsible for the initiation and maintenance of the low oxidation-reduction potential, but their identity is still unknown. Xanthine oxidase, which is very active in *Tineola* larvae, functions at, and assists in maintaining, a very low redox potential, but its distribution in the body suggests that it cannot play an important part in keratin breakdown. This conclusion is strengthened by its weak activity in dermestid larvae which also maintain reducing conditions in the midgut [Irzykiewicz (54)]. Urea, which is well known for its denaturing effect on some proteins, is present in the *Tineola* midgut but in too low concentration (a maximum of 0.5 M) to produce any large effect on wool digestion [Powning (93)].

Day (20) recorded the sudden marked decrease in birefringence which occurs when wool passes into the middle region of the midgut of *Tineola* larvae. The hemicylindrical orthocortex of the wool fiber is digested first, leaving the more resistant paracortex and scales to be degraded or excreted according to the rate at which the larva is feeding [Mercer (76)]. The middle half of the midgut differs from the anterior and posterior quarters in having a columnar epithelium interspersed with few flask-shaped goblet cells, compared with a fairly regular alternation of columnar and cigar-shaped goblet cells. Waterhouse (120) showed that the digestive juices of the middle half have a lower redox potential than those elsewhere in the gut. He also

found (118) that, when woolen fabrics impregnated with salts of elements which form insoluble sulphides are ingested, characteristically coloured sulphides are formed, often appearing abruptly as the wool enters the mid midgut. These sulphides are probably produced from hydrogen sulphide liberated from cysteine by a very active desulphydrase shown by Powning (94) to be present in the midgut. Much of the sulphide is excreted, but some is accumulated in the goblet cell cavities where it remains until the midgut epithelium is cast off and regenerated at the next moult. It appears that, in the presence of amino acids and polypeptides liberated during digestion, freshly produced sulphides form extremely finely dispersed colloidal suspensions which are capable of being taken up through cell membranes [Waterhouse (118)]. The goblet cells in the various regions of *Tineola* behave differently towards sulphides. Their goblet cavity does not appear to open on to the lumen. The function of the goblet cell in storage excretion appears to be normal, since evidence for this was also found in other lepidopterous larvae [Waterhouse (119)]. *Tineola* fed on these metal-impregnated fabrics excrete much less cystine than on untreated wool, and it is evidently unwanted sulphur which is used to produce the sulphides. Elements which do not form insoluble sulphides do not produce coloured accumulations in the goblet cells. However, alkaline earths are deposited as granules (mainly as phosphates) principally in the columnar cells of the anterior midgut, and it is possible that small quantities of absorbed fluoride are deposited with calcium in these granules [Waterhouse (118)].

A basic similarity in all wool-digesting insects so far examined is the possession of a low oxidation-reduction potential in the midgut digestive juices. Evidently related to this is the fact that the midgut is far less thoroughly tracheated than usual [Day (21); Waterhouse (121, 122)]. Thus the oxidation-reduction potential of the poorly tracheated midgut of several dermestid larvae falls in the range -109 to -230 mv., at a pH of about 7.0. Wool is rapidly reduced under these conditions as indicated by an intensely positive nitroprusside reaction produced by breakage of disulphide bridges and the formation of sulphydryl groups. Unlike *Tineola*, the dermestid midgut is not differentiated into zones, nor are sulphides formed following ingestion of appropriate metals [Waterhouse (121)]. It is not surprising, therefore, that only a very weak cysteine desulphydrase occurs in these larvae and that their excreta therefore contain more cystine than those of *Tineola* [Powning (93)]. A wide range of ingested metals are complexed with cysteine in the dermestid midgut, but this process generally results in no colour change. The most typical colour change follows the ingestion of cobalt-impregnated fabrics, when the characteristic, brown, water-soluble, cobaltic-cysteine complex is produced [Waterhouse (121)].

Bird-infesting Mallophaga digest feather keratin in a fashion similar to that of carpet beetle larvae. Waterhouse (122) found that they have slightly alkaline (about pH 8.0), reducing (about -200 mv.) digestive juices and produce metal-cysteine complexes, but not sulphides. However, the mammal-

infesting Mallophaga have neutral or acid digestive juices and these are oxidising, not reducing. Contrary to many textbooks, neither hair nor wool is regularly present in the digestive tract, and these species appear to ingest mainly epithelial debris and skin secretions. Free sulphydryl groups were not detected in the gut lumen, and it is doubtful whether keratin could be digested during passage through the alimentary canal.

DIGESTION OF WAX

Larvae of two species of moth (*Galleria mellonella* Linnaeus and *Achroia grisella* Fabricius) normally live on honeycomb which contains some 40 per cent of beeswax. Although some of the constituents of the wax may provide a valuable source of energy and of water, *Galleria* thrives on artificial diets lacking wax [Haydak 46, 47); Good *et al.* (41)] indicating that its nutritional requirements are not unusual. The composition of beeswax is not known with any certainty, and most of the data is based on analyses performed many years ago using what are now known to be inadequate methods. It is said that yellow beeswax contains some 70 per cent esters (33 per cent is myricyl palmitate), 13 per cent wax acids, and 12 per cent hydrocarbons [Warth (114)]. It is also known that hydroxypalmitic acid is present [Toyama & Hirai (112)]. About 50 per cent of the wax ingested by *Galleria* does not appear in the excreta [Dickman (27); Niemierko & Wlodawer (83); Wlodawer (136)], but no assessment is yet available of the relative importance of the larval enzymes and of bacteria in wax breakdown. Preliminary indications are that sterile larvae can digest a high proportion of stearic acid, hexadecyl alcohol, and octadecyl stearate incorporated in their diet, but make relatively little use of the C 30 paraffin *n*-triacontane [Waterhouse (126)].

Lipolytic bacteria can readily be cultured from the alimentary tract and these utilize principally the fatty acids, although esters are also attacked [Dickman (27); Florkin *et al.* (38); Rybicki (103)]. Rybicki (103) reared a few larvae aseptically on sterile comb, although growth was much slower than usual and development seldom proceeded to completion, and Wollman (137) also reported growth under sterile conditions. However, the fate of the wax constituents under these conditions is unknown, and it may well be that bacteria normally present in the gut rectify dietary deficiencies other than those associated with breakdown of wax.

A lipase [Duspiva (28); Fiessinger & Gajdos (34); Metalnikov (75)], a lecithinase, and a cholesterolesterase [Clément & Frisch (14)] are present in extracts of nonsterile larvae. In spite of several early reports to the contrary [Dickman (27); Duspiva (28); Florkin *et al.* (38); Kraut *et al.* (58)] larval extracts are also able to hydrolyse beeswax, although less rapidly than tributyrin and ethyl *n*-butyrate and about as rapidly as olive oil [Good *et al.* (41); Mankiewicz (70, 71); Pertzoff (87)]. However, these lipolytic enzymes may, like the chitinase of snails [Jeuniaux (55)], be secreted by the bacterial flora and not by the larvae. The claim that the lipolytic enzymes differ in

larvae reared on natural and artificial diets [Good *et al.* (41)] requires substantiation.

Niemierko & Wlodawer (82, 83) and Wlodawer (136) have obtained valuable information on wax utilisation by nonsterile larvae feeding on comb. One milligram of larval lipoid was produced from ingestion of 16.1 mg. beeswax, of which 9.5 mg. was unsaponifiable material and 6.6 mg. fatty acids. More unsaponifiable material (5.0 mg.) than fatty acids (3.0 mg.) disappeared, 4.3 mg. unsaponifiable material and 2.8 mg. fatty acids were excreted, and the remainder of each was present in the larval body. It is suggested that the unsaponifiable substances (principally alcohols) are oxidised to fatty acids and that these partly replace fatty acids which are continually being degraded. Evidence was also obtained that some of the hydrocarbons of beeswax disappeared during passage through the alimentary tract, as also did some of a small quantity of paraffin wax added to the diet [Niemierko & Wlodawer (83)]. However, further work using pure materials is required to provide more precise information on utilisation of individual constituents. Most of the inorganic phosphate, in which excreta of *Galleria* and *Achroia* is very rich, is metaphosphate instead of orthophosphate as in many other insects [Niemierko & Niemierko (81)], but whether this is of significance in relation to wax digestion is unknown [Niemierko *et al.* (84)].

DIGESTION OF COLLAGEN

Ziffren *et al.* (141) and Waterhouse & Irzykiewicz (127) have recently confirmed the early report of Hobson (49) that a collagenase was present in the excreta of sterile blowfly larvae. A collagenase has also been reported in larvae of the warble fly (45). No collagenase activity was found in protease preparations from *Musca, Periplaneta, Locusta, Tineola,* or *Bombyx* [Waterhouse & Irzykiewicz (127)]. A true collagenase is produced by some, but not all, members of the bacterial genus *Clostridium* [MacLennan *et al.* (68)], but adequate evidence for the presence of this enzyme in any animals other than fly larvae is not yet available.

INVERTASE

The occurrence of invertase in many insects is well known, but it is only very recently that the insect enzyme has received any detailed attention by modern methods [White & Maher (130, 131); Gray & Fraenkel (42, 43); Täufel & Müller (110); Wolf & Ewart (138, 139)]. During the enzymatic degradation of sucrose into glucose and fructose it is now well established that the hexose residue split from sucrose by the action of invertase is not transferred exclusively to water (as in a chemical hydrolysis) but that other sugars present in the reaction mixture may act as acceptors. In this way various oligosaccharides are produced as intermediate products. Yeast invertase is a so-called fructo-invertase (or fructofuranosidase), since the fructose end of the molecule is attacked and is involved in the subsequent production of oligosaccharides [White (129)]. On the other hand, insect invertases, like

those of higher animals, are all believed, with the exception of one claim [Bealing (6)], to be gluco-invertases or glucosidases, and the most characteristic trisaccharide formed (which is known as gluco-sucrose or fructo-maltose) contains two glucose and one fructose molecules. This sugar has been identified in coccid and aphid honeydew, in honey, and in the excreta of a blowfly fed on sugar [Duspiva (29, 30); Gray & Fraenkel (42, 43); Wolf & Ewart (138, 139); White & Maher (130)]. Gray & Fraenkel (42) suggested that this trisaccharide may be expected to be formed in the digestive tract of any insect which possesses invertase and ingests sucrose. This generalisation is not strictly true, however, since *Icerya purchasi* Maskell contains an invertase (apparently acting only on sucrose) which produces melezitose, glucose, and fructose but no gluco-sucrose [Wolf & Ewart (138)]. Wolf & Ewart (139) have suggested the name gluco-sucrose instead of fructo-maltose, since in *Coccus hesperidum* Linnaeus it forms the first of a series (gluco-, malto-, maltotrio-, and maltotetro-sucrose) produced by the insect by the progressive addition of 1, 2, 3, or 4 glucose units to the glucose end of the sucrose molecule.

Weak fructosidase activity was shown in honey invertase by the slight hydrolysis of raffinose to melibiose [White (129)], but it is not entirely certain that this activity is derived from the honey bee rather than from microorganisms contaminating the honey. A reinvestigation of the position using aseptically reared insects is required before a definite statement can be made.

HONEYDEW

For many years honeydew has been regarded as the result of the need of certain plant-sucking insects to obtain an adequate supply of nitrogen in which plant sap was allegedly deficient. In the process of obtaining nitrogenous materials excess carbohydrates and water were ingested, and these were excreted as honeydew with the aid of the filter chamber, a modification of the gut which is present in varying complexity in many honeydew producers.

It has now been shown that the honeydew of the pineapple mealybug [Gray (44)] and of various aphids [Auclair & Maltais (1); Lamb (62); Maltais & Auclair (69); Mittler (79)] contains relatively large amounts of up to 20 different amino acids and amides, some of which were not present in the sap ingested. In the lily aphid the dry honeydew contains 13.2 per cent amino acids and 35.7 per cent sugars. Lindemann (65) found that nitrogen, and Mittler (79) that not only nitrogen but also all amino acids, were in a higher concentration in the phloem sap than in the honeydew. There did not appear to be any differential utilisation of amino acids by the aphid [Mittler (79)]. The free amino acids in the honeydew appeared to come directly from the ingested sap and not to arise as products of protein breakdown or by the fixtion of atomospheric nitrogen. It appears, therefore, that the content of free amino acids and amides in sap is usually considerably in excess of the aphids' requirements.

The same carbohydrates as in the sap of the host plant (fructose, glucose, sucrose, glucose-1-phosphate) were present in the honeydew [Gray (44); Gray & Fraenkel (42, 43); Duspiva (30] and often, in addition, gluco-sucrose formed by the action of insect gluco-invertase [Duspiva (30; Gray & Fraenkel (42, 43); Wolf & Ewart (138, 139)]. However, in *Icerya purchasi* [Wolf & Ewart (138)] and *Eucallipterus tiliae* (Linnaeus) [Bacon & Dickinson (2)] melezitose is the principal additional sugar present. Adonitol and dulcitol (102), sorbitol and inosital [Duspiva (30)] are present in some honey-dews, as are also uric, malic, and citric acids. Honeydew must, therefore, be regarded as a mixture of excess carbohydrates, amino acids, water, and other sap constituents to which the insect contributes various additional metabolic and excretory products.

In view of the foregoing, it would, therefore, seem to be necessary to postulate that some necessary substance in short supply other than amino acids and sugars is obtained through the action of the filter chamber. An alternative and more attractive hypothesis is that the turgor pressure in the plant tissue largely maintains the flow of sap through the stylets into the alimentary canal of the insect so that, once a suitable source of sap is tapped, ingestion is a relatively passive process. Kennedy & Mittler (56) cut off the mouthparts of the willow aphid without dislodging the stylet tips from their normal situation within a phloem sieve tube. They found that sap exuded continuously from the stylet stump at the rate of about 1 cmm. per hr. and that similar rates of honeydew excretion were recorded from adjacent in-tact aphids. If this situation proves to be general, the function of the filter chamber may be to bypass much of the water of this continuous flow of fluid, thereby reducing not only the volume, but also the rate of flow of material through the region of the midgut where digestive enzymes have to function. There is no doubt that a satisfactory technique for feeding aphids and coccids through an artificial membrane would greatly assist in obtaining an understanding of many aspects of honeydew production.

CONTROL OF ENZYME SECRETION

Very little is yet known concerning the stimulation or control of enzyme secretion. The production of digestive enzymes is probably a continuous process in many insects, since the alimentary canal, especially in larvae, is often kept full of food except at moulting. However, in predaceous, blood-sucking, and other insects which feed intermittently, some means of con-trolling enzyme secretion might be expected. In vertebrates three mechanisms co-exist; (a) the stimulation may be nervous and arise via sense organs in the head, (b) the food or its products may stimulate secretion directly, or (c) the stimulus may be brought about by the increased production of gastrointestinal hormones, such as secretin or gastrin, which are elaborated by the alimentary tract.

There are certain broad generalisations in experiments with insects which

have involved adult Orthoptera [Day & Powning (23); Schlottke (104, 105)], Coleoptera [Schlottke (104, 105)], and Diptera [Champlain & Fisk (13); Fisk (35); Fisk & Shambaugh (36, 37); Shambaugh (107)]: (*a*) Enzyme activity is diminished by starvation. (*b*) Enzyme activity often falls temporarily below the starvation level when food is again taken. (*c*) Brief feeding generally results in a gradual increase in enzyme activity, continuing for some hours. (*d*) Secretion of all enzymes tends to be stimulated irrespective of food [Day & Powning (23), Schlottke (104, 105)]. However, in *A. aegypti*, whereas blood feeding stimulated both protease and invertase, sucrose feeding produced little or no effect on either [Shambaugh (107); Fisk & Shambaugh (37)]. Similarly in *Stomoxys* protease activity only showed a slight temporary increase after ingestion of sucrose solution [Champlain & Fisk (13)].

In *A. aegypti* there is a strong positive correlation between amount of food ingested and protease activity. Furthermore, ingestion of different blood fractions had different effects on the subsequent level of protease activity [Shambaugh (107)].

A direct neural stimulus is reputedly not involved in these processes since all responses observed are delayed ones and since, in the cockroach at least, the nerves innervating the midgut appear to be motor and to supply only the gut musculature [Day & Powning (23)].

It is not improbable that both endocrine and direct food stimulation of secretion occurs, although it is only for the former that there is, as yet, even suggestive evidence. Thus endocrine influence is suggested by the increased number of mitoses in the midgut epithelium of *Tenebrio* after the injection of blood from fed into starved beetles [Day & Powning (23)]. Furthermore, in *Periplaneta* the caeca are said to commence secretion before the midgut [Schlottke (105)], although their long narrow form is ill-fitted for the rapid diffusion of materials from the gut lumen to their closed ends [Day & Powning (23)]. If the stimulating material is hormonal it would appear, therefore, that it is not produced by the midgut itself. It is perhaps relevant to note that Cameron (11) has reported a pronounced effect on gut peristalsis rate of an aromatic phenolic compound (not epinephrine) extracted from, and evidently produced by, the corpora cardiaca of *Periplaneta*. However, in *Calliphora*, Thomsen (111) believes that the neurosecretory material observed in the nervi oesophagi (which pass from the corpus cardiacum and hypocerebral ganglion to the gut) originates from the median neurosecretory cells of the brain. Whether or not these particular materials influence secretion of digestive enzymes, the anatomical requirements for such an influence have been shown to exist. Cholinesterase is believed to be a component of the mechanism enabling active transport of ions in *Chironomus* [Koch (59)], and it is therefore of interest to note that acetylcholine affects the tonus and rate of contraction of the alimentary canal [Cate (12); Kooistra (57)].

HUNGER REACTION

Bolwig (8) has carried out one of the few studies of hunger reaction. Bodyless housefly heads made drinking movements when their proboscis came in contact with sugar solution. Flies with their proboscis forcibly in touch with solution continued to pump even when the crop was full, only contra pressure preventing further intake. The pumping reaction of the fulcrum appears, therefore, to take place independently of the hunger or thirst condition of the fly. The removal or ligaturing of the unfilled crop did not abolish the hunger reaction, nor did the removal of the full crop or entire abdomen from satiated flies induce hunger signs for an hour or two. It is apparently not the contents of the crop which determines whether or not a fly shows hunger.

Contrary to an early claim [Marchal (72)] for *Dytiscus*, destruction of the frontal ganglion does not appear to influence the hunger reaction or to inhibit swallowing. However, ligaturing of the oesophagus of fed flies just in front of the proventriculus (to destroy the nerve connections from midgut to brain) resulted in a resumption of hunger reactions at full strength, and these flies seemed to be unable to satisfy their hunger. Inhibiting impulses seem thus to be conducted from the midgut to the brain or related organs soon after a small amount of sugar solution has passed through the proboscis. The hunger condition, is, however, maintained for some time after a meal. Thus newly-fed flies still turned towards a droplet of sugar solution when touching it with their front tarsi, but, however, without drinking. After some time this reaction ceased. Bolwig suggests that the hunger reaction is caused by the effect of the exhausted haemolymph on the nervous system. This he supported (9) by results indicating a correlation between the degree of thirst and the osmotic pressure of the haemolymph. Such a hypothesis might also explain the hunger reaction for blood of *Stomoxys* and *A. aegypti* which had crops full of sugar solution (see below). It is evident, however, that further work on this problem is required, for in the adult blowfly the alteration of the osmotic pressure of the blood by injection of sugar solutions does not affect the threshold of the labial chermoreceptors to sugar solutions [Dethier (26)].

FOOD DISTRIBUTION IN BLOOD SUCKING DIPTERA

It has long been known that blood and nectar go initially to different destinations in the mosquito gut. Blood, irrespective of the manner in which it is fed, passes in both male and female mosquitoes principally to the midgut. In some species practically none reaches the diverticula (the crop consists of two dorsal and one ventral diverticula), whereas in other species they may receive a considerable amount of blood [Trembley (113)]. On the other hand, sugar solutions go primarily to the ventral diverticulum, small amounts passing at intervals to the midgut. From experiments in which sugars were added to blood or blood fractions it is clear that both whole blood and various sugars are detected and that the relative concentrations

of the components of a mixture determine its destination [Bishop & Gilchrist (7); Day (22); Fisk (35); Hosoi (50); Trembley (113)]. The particulate nature of blood is of significance, for saline-suspensions of red cell ghosts or of sarcosomes from blowfly thoracic muscles went mainly to the midgut. Day (22) suggests that the four types of sense organ recorded many years ago [Sinton & Covell (108); Barrand & Covell (5)] in the buccal cavity of the mosquito distinguish the various food components and transmit impulses via the stomatogastric nervous system to cause the contraction of sphincter muscles either of the diverticula or the proventriculus.

Tabanids [Cragg (15); Olsufiev (85)], *Phlebotomus* [Mukerji (80)], and the stable fly *Stomoxys* [Kuzina (60); Lotmar (67)] also divert blood to the midgut and sugar solutions to the crop, whereas *Glossina* sends blood to both regions [Lester & Lloyd (63); Wigglesworth (132)]. The factors determining distribution in *Stomoxys* are, perhaps, slightly different from *A. aegypti* for both serum and sugar solutions thicknened to the viscosity of blood with agar or gelatin went, in different individuals, to the crop only, to the midgut only, or to both. Sense organs have not been described in *Stomoxys*, but it is interesting to note that cibarial organs occur in *Drosophila* [Hertweck (48); Miller (78)]. The hunger reaction of *Stomoxys* [Lotmar (67)] and *A. aegypti* (126) is not satisfied by a crop full of sugar solution for the former filled the midgut immediately and the latter within a few hours when blood was offered under these circumstances.

Since the crop is impermeable to water, Denisova (24, 25) believes that it plays an important part in water economy, because of the fact that water is rapidly eliminated from other regions of the gut. Day (22) suggests that since a crop full of nectar does not preclude the taking of a blood meal should this become available, this arrangement would be of great survival value when hosts are only encountered occasionally.

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SOME ASPECTS OF INTERMEDIARY METABOLISM OF CARBOHYDRATES IN INSECTS[1,2,3]

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The field of insect intermediary metabolism has lagged far behind comparable research in vertebrates and microorganisms. However, the recent entrance into this field of study by individuals whose primary research interest is general physiology or biochemistry per se has meant rapid progress in this area within the past few years. For example, in an early review on insect physiology, an apologetic note is made by the authors for the virtual absence of information on the nature of the processes involved in insect metabolism as well as their sites of occurrence [Hoskins & Craig (1)]. As late as 1950, an authoritative text on comparative physiology contained only a rare allusion to insect intermediary metabolism [Prosser (2)], whereas two excellent contemporary textbooks on insect physiology include only a handful of references on this subject dated since 1940, most of which deal with data on studies of respiratory metabolism in relation to development and metamorphosis [Chauvin (3); Wigglesworth (4)]. The intensification of an active interest in insect intermediary metabolism is attested to by the increasing number of scattered references between 1940 and 1950 (some still "unpublished" at the time of printing) in the most recent treatise on insect physiology [Roeder (5)].

In attempting to present a critical review which will summarize the present status of this subject, the author has found it desirable to re-present older findings on occasion, in order to indicate their current significance in and relationship to a rapidly emerging, coherent pattern of the metabolic processes in insectan tissues. However, the emergence of such an integrated picture has resulted chiefly from studies reported upon during the past few years.

In considering the intermediary metabolism of carbohydrates[4] specifically, the author has deemed it desirable to include not only work on the

[4] We shall include under the term "intermediary metabolism" all changes occurring between the time of entry of carbohydrates into the organism (usually as the hexose sugar) and the moment of discharge of the final chemical products.

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various known aerobic and anaerobic phases thereof but also certain aspects of phosphorus metabolism, known to be related to (if not dependent upon) such carbohydrate metabolism. An early review on the chemical changes during metamorphosis includes coverage of the earlier literature on the role of glycogen metabolism in insect development specifically [Needham (6)], followed by a more recent review by the present writer on glycogen metabolism [Rockstein (7)]. Such literature reflected the continuing interest of biologists in establishing a chemical basis for the remarkable visible changes which accompany insect development, especially metamorphosis; its relevancy to this review is the fact that it established glycogen as an important storage source of energy in a variety of insect species [Vaney & Maignon (8); Ronzoni & Bishop (9); Hill (10); Blanchard & Dinulescu (11, 12); Levenbook (13, 14)]. Similar information has been obtained from comparable studies on the respiratory metabolism of, and related biochemical bases for flight in *Drosophila repleta* Wollaston by Chadwick & Gilmour (15), in *Drosophila melanogaster* Meigen by Wigglesworth (16), in *Drosophila funebris* (Fabricius) and *Phaenicia sericata* (Meigen) by Williams, Barness & Sawyer (17), in *Tabanus* spp. by Hocking (18), and in *Culex pipiens* form *berbericus* Roubaud by Clements (19). Such experiments have revealed (*a*) the primary importance of glycogen as the chief source of energy for flight in a number of species (and that flight ability may be directly related to the extent of glycogen reserves in prolonged flight or as a function of age) and (*b*) that anaerobiosis is an important component in the complex intermediate processes which energize flight, just as it is known to be in the metabolism of vertebrate muscular activity.

THE EMBDEN-MEYERHOF CYCLE

What was once considered a direct oxidative process involving the combination of simple carbohydrates (like glucose) with oxygen to form carbon dioxide and water has gradually been extended during the past 25 years to encompass a complex series of linked, mostly reversible processes, during the course of which is trapped and stored the energy which will drive the numerous endergonic processes, important in such biological activities as muscle contraction, nerve impulse conduction, and bioluminescence. Collectively the initial anaerobic phases of this complex metabolic pathway is referred to variously as glycolysis, anaerobic glycolysis, or the Embden-Meyerhof cycle and includes the (mostly reversible) processes by means of which glycogen or glucose may be degraded to the level of lactic acid without the intervention of processes requiring molecular oxygen as a reactant.[5]

Early information as to the occurrence of this cycle in insects came chiefly from studies of respiratory function as a possible reflection of a kind of

[5] Readers desiring further orientation may want to consult Chapter 5 of Roeder (5) which contains an excellent, short introduction to the entire subject of intermediary carbohydrate metabolism in higher animals and microorganisms, or any good recent textbook of biochemistry for a more advanced treatment of the subject.

metabolism, such as oxygen uptake before, during and after anaerobiosis and (in some) concomitant changes in compounds like glycogen, glucose, and lactic acid. In *Periplaneta americana* (Linnaeus), for example, the amount of lactic acid produced is not sufficient to account for all the glycogen consumed (and therefore suggests other anaerobic carbohydrate metabolic pathways than glycolysis) [Davis & Slater (20, 21); Slater (22)]. Typical anaerobiosis (with typical oxygen "debt" and "repayment") was described for male nymphs of *Melanoplus femur-rubrum* (DeGeer) and *Melanoplus differentialis* (Thomas); concomitant fall in blood pH with build-up of oxygen debt strongly suggested typical glycolysis in these species [Bodine (23)]. Blanchard & Dinulescu (11, 12), in reporting an inverse relationship between lactic acid formed and glycogen consumed during development of (third instar) *Gasterophilus intestinalis* (DeGeer) (as well as during starvation) under anaerobic conditions (N_2 atmosphere), found discrepancies similar to those reported earlier by Davis & Slater (20, 21, 22). Gilmour's confirmation of their findings in *Tenebrio molitor* Linnaeus, included his suggestion that the high O_2 uptake combined with a low R.Q. during recovery from anaerobiosis meant an additional anaerobic pathway with fatty acid formation (24). Yet, in an earlier paper, Gilmour had (probably prematurely) inferred from respiratory data of a similar anaerobiosis experiment with *Cryptocercus punctulatus* Scudder that "lactic acid glycolysis" was the sole anaerobic metabolic process (25); a similar inference was drawn by Gilmour for isolated hind femoral muscles of *M. femur-rubrum* and *M. differentialis* (26). In a later study, Agrell (27) confirmed Gilmour's findings [in *T. molitor* (24)] of discrepancies between glycogen consumed and lactic acid produced, in developing *Calliphora erythrocephala* (Meigen).

Of greater significance to the entire field of carbohydrate metabolism and comparative biochemistry was the isolation from arthropod (crustacean) muscle of argininephosphoric acid the invertebrate analogue of the vertebrate phosphagen, creatinephosphoric acid, by Meyerhof & Lohmann (28, 29, 30), soon followed by the demonstration of the reaction between phosphagen and ADP for the first time in minced crustacean muscle by Lohmann (31) and Lehmann (32). Shortly thereafter, Baldwin & Needham reported concentrations of argininephosphate in the muscle from ground thoraces of *Calliphora* and *Lucilia* to be of the same order of magnitude as frog heart muscle creatinephosphate, with adenyl and inorganic pyrophosphate of similar concentrations in fly muscle to those of frog or rat skeletal muscle (33). It remained for Albaum & Kletzkin (34) to establish conclusively the presence in *D. melanogaster* adults of an ATP with the same physical, chemical, and physiological properties as vertebrate ATP. Albaum also demonstrated its presence in four other insect species, including *T. molitor* larvae (35). In establishing the presence of high energy phosphates in insect tissues, their report (33) can be considered a milestone in the many steps which remained to be taken before the precise metabolic pathway for carbohydrates could be established in insects. Calaby (36) confirmed their findings

for *D. melanogaster* and also showed that the ATP in hind femoral and thoracic muscles of *Gastrimargus musicus* (Fabricius) is identical with that from rabbit muscle.

Although occasional studies on the mode of action of insecticides in relation to insect metabolism helped to establish the probable existence of the glycolytic pathway in *P. americana* [Merrill, Savit & Tobias (37)] and in *Carpocapsa pomonella* (Linnaeus) [Graham (38)], it was the combined efforts of an enzyme chemist and a zoologist which established many important facts concerning such a pathway in insects (and gave important clues to the existence of an oxidative pathway as well) [Barron & Tahmisian (39)]. They reported that teased leg muscles from *P. americana* showed glycolysis with end products other than lactic acid [data reminiscent of Gilmour's earlier respiratory metabolic studies (24)], and that iodoacetate failed to inhibit lactic acid production, as it does in vertebrate tissues. Their failure to find pyruvic acid production in the American cockroach during glycolysis was negated by the report by Humphrey (40) not only of the normal presence of both pyruvic acid and lactic acid in American cockroach muscles (homogenates, however, in this case) but also the production of both acids during glycolysis; this was also shown to be true for *Locusta migratoria* Linnaeus wing muscle homogenates or extracts [Humphrey & Siggins (41)]. They also confirmed the earlier reported deviation by insects from vertebrate muscle glycolysis (39) that iodoacetic acid failed to inhibit either lactic or pyruvic acid production during glycolysis by the muscles of either of these two species. An additional, important step toward establishing the presence of a vertebrate-like glycolytic pattern in insect tissue was the ability to enhance locust muscle glycolysis by the addition of known intermediates of the vertebrate glycolytic system; glucose-1-phosphate, glucose-6-phosphate, and fructose-1,6-diphosphate. However, it remained for a very recent study to present conclusive stepwise evidence for the existence in total homogenates of adult (male) house flies, *Musca domestica* Linnaeus, of glycolytic pathways identical with those observed for vertebrate tissues, by Chefurka (42), who demonstrated the presence of the essential enzymes of the vertebrate glycolytic system; viz., hexokinase, isomerase, phosphofructokinase, aldolase, phosphotrioseisomerase, alpha-glycerophosphate dehydrogenase, phosphoglyceraldehyde (triosephosphate) dehydrogenase (with DPN as coenzyme), enolase, and lactic dehydrogenase. Like the vertebrate enzymes of this group, all were found in the soluble fraction of the homogenate and were therefore extramitochondrial. He also demonstrated that house fly glycolysis is of the phosphorylative type, with all phosphorylated intermediates of the Embden-Meyerhof system being metabolized by such homogenates, although added hexosediphosphate and appropriate inhibitors were necessary (under otherwise suitable conditions) to maintain a high level of glucose phosphorylation. Finally, he found that iodoacetic acid failed to inhibit house fly phosphoglyceraldehyde dehydrogenase, which confirmed and explained the basis for earlier reports (39, 40, 41) of the failure of iodoacetate to inhibit pyruvic

or lactic acid production by orthopteran muscle. Recently, Faulkner was unable to demonstrate the presence of either phosphoglucomutase or phosphorylase with either glucose or glycogen in fifth instar larval blood of the silkworm, *Bombyx mori* (Linnaeus), but he obtained evidence for direct attack upon glucose-1-phosphate by an acid phosphatase, not activated by Mg ions, but inhibited by arsenate and phosphate and competitively inhibited by F ions (43). It is obvious that an exhaustive study of silkworm intermediary metabolism is desirable along the lines of Chefurka's study in the house fly (42).

Sacktor's more recent but timely report presents additional evidence for the existence of a glycolytic scheme in house fly muscle homogenates in terms of the ability of such preparations to oxidize a considerable number of compounds, including the intermediates of the vertebrate glycolytic (and Krebs) cycles (44). Unlike Chefurka (42) who had found that TPN and DPN might be employed for the activity of certain of the enzymes involved in glycolysis, Sacktor found that flight muscle homogenates required the addition of neither TPN or DPN for the oxidation of glucose under otherwise appropriate conditions. Sacktor's findings that phosphopyruvate could be directly metabolized (and at a rate comparable with Krebs cycle intermediates) suggested to him the existence of an alternative mechanism for by-passing the later stages of glycolysis directly from phosphopyruvate. (This is in keeping with some of the above-discussed hypotheses of Gilmour (24) and of Barron & Tahmisian (39) based on discrepancies in glycogen consumption:lactic acid formation ratios.)

The recent application of paper chromatographic and radiometric techniques by Winteringham & Hellyer (45) and Winteringham, Bridges & Hellyer (46) helped to show the ease of incorporation of (radioactive) P into a variety of acid-soluble compounds, including ATP, ADP, and phosphagen, by house fly thoracic homogenates. This suggests a likely technique already in the employ of biochemists which should prove useful in desirable correlative studies of substrate presence, enzyme activity, and metabolites produced in this and related areas of insect intermediary metabolism.

In further clarifying the picture of glycolytic metabolism by house flies, Chefurka's recent, brief communication (47) has also confirmed the existence of the "hexosemonophosphate shunt"[6] in (female) house fly thoracic homogenates, in showing activity of glucose-6-phosphate dehydrogenase,

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[6] This is an alternative oxidative pathway demonstrated to exist in bacteria and higher animals, by which glucose-6-phosphate is oxidized [to phosphogluconic acid, then to ribose-5-phosphate (by oxidation and concomitant decarboxylation) and ultimately] to sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate. The latter two can be reconverted to the hexose, fructose-6-phosphate. In higher animals this is thought to be the major pathway for glucose utilization in the liver, whereas the corresponding predominant system in muscle is the (Embden-Meyerhof) glycolytic pathway.

6-phosphogluconate dehydrogenase and transketolase, as well as the expected pentose, triose, heptulose, and hexose compounds.

THE KREBS CITRIC ACID CYCLE

Although glycolysis results in the formation of some energy depots, active cells typically depend for most of their energy upon oxidative conversion to carbon dioxide and water of the pyruvate derived from such glycolysis. Such energy is trapped and stored in the form of labile energy-rich compounds like ATP, through a series of reversible dehydrogenation and oxidative steps, collectively called the Krebs citric acid cycle. This begins with the formation of an active (decarboxylated) two-carbon fragment and terminates in the final transfer of protons to oxygen, as water is resynthesized. It is this still incompletely identified system which is, in fact, responsible for most of the oxygen consumed and carbon dioxide released by active, respiring cells.

Our recognition of the presence of a Krebs cycle in insects originates in the early, important report by Barron & Tahmisian (39) that known intermediates of the vertebrate Krebs cycle, alpha-ketoglutarate, malate, succinate, and citrate, enhanced O_2 uptake by American cockroach muscle and that malonate inhibition thereof was reversible by fumarate addition. Later, succinic acid was identified in the pupa of *Phaenicia sericata* (Linnaeus) by Pryor, Russell & Todd (48) and in *Gasterophilus intestinalis* (DeGeer) larval blood by Levenbook & Wang (49). As for enzymes of this system, Roeder *et al.* (50) found the succinic dehydrogenase in *P. americana* femoral muscle to be five times more active than that known for frog muscle homogenates. Recently, high titers of such related enzymes as pyruvic, malic, and succinic dehydrogenases (as well as succinoxidase and cytochrome oxidase) were described for isolated sarcosomes (giant mitochondria) from the blow fly *Phormia regina* (Meigen) by Watanabe & Williams (51). In a study of the utilization of metabolic energy during metamorphosis, Agrell found that pupal blood and other tissues of *C. erythrocephala* could reduce methylene blue with the addition of the Krebs intermediates fumarate, malate, or succinate (as well as acetate) (27, 52 to 55). In a nicely integrated fashion, he also noted a reciprocal relationship between glycolytic and Krebs cycle activity during metamorphosis, both in terms of changes in initial and final concentrations of carbohydrates as well as of the relative effectiveness of inhibitors of each of these pathways (fluoride and malonate, respectively) at different periods of development.

A most important report (unfortunately not known to be published in full form) indicated that such known Krebs cycle component enzymes as aconitase, isocitric, malic, and succinic dehydrogenases and condensing enzyme occur in unwashed homogenates, water extracts and even acetone powder extracts of mature *D. melanogaster* larvae [Spirtes (56)]. He also reported as present the peripherally-related enzymes cytochrome oxidase and oxalacetic oxidase. Collias, McShan & Lilly (57) found succinic and

malic dehydrogenases (and cytochrome oxidase) in total body homogenates of adult *Oncopeltus fasciatus* (Dallas) and noted that succinic dehydrogenase was inhibited by malonate (as in mammalian tissues). Sacktor (58) also found malic dehydrogenase plus a number of important related enzymes and coenzymes in house fly sarcosomes. In a later study (discussed earlier in this review), Sacktor (44) found that all Krebs cycle intermediates could be oxidized by the particulate fraction alone of house fly thoracic muscle homogenates. In a study on oxidative metabolism of *L. migratoria* wing muscle sarcosomes, Rees (59) inferred the presence of a (glycolytic and) Krebs cycle from the ability of added (glycogen or) citric acid intermediates to enhance or stabilize normal respiration. Sacklin, Terriere & Remmert (60) also demonstrated the participation of pyruvate, succinate, and citrate in the oxidative activities of house fly homogenates (in the presence of suitable cofactors, ATP, DPN, TPN, and cytochrome-*c*). As an important corollary finding, Wolff & Williams (61) recently identified coenzyme A in developing larvae of *Platysamia cecropia* (Linnaeus); this supplies to our knowledge of insect metabolism another important link in the chain of chemical events analogous to those already established for higher animals.

OXIDATIVE METABOLISM AND PHOSPHORYLATION

It has already been indicated that scattered studies involving respiratory measurements under a number of different conditions have given strong clues as to the presence of a glycolytic and oxidative metabolic cycle in insects. Such studies require, however, the obtaining of correlated data on changes in concentrations of metabolites as well as on the presence of appropriate enzyme systems. Indeed, such a rather extended study has been made by Agrell (27, 52 to 55, 62, 63); in addition to indicating how metabolic energy is utilized during metamorphosis, this study has also supplied well-correlated biochemical and histological grounds for the division of pupal development of *C. erythrocephala* into three distinct stages: histolysis, histogenesis, and differentiation. In the same connection, Zebe has recently applied classical methods of respiratory metabolism to the energetics of flight in a considerable number of lepidopteran species (64); from R.Q. values for such insects at rest and during flight he inferred that Lepidoptera utilize fat during rest or in flight, even though they feed on nectar (!).

As we saw above, the benefit to the cell from carbohydrate metabolism lies in the entrapment of energy at several points along the complex pathway of anaerobic and aerobic processes into labile, energy-rich phosphorylated compounds. However, the ultimate end of aerobic or oxidative metabolism is now known to be mediated through a series of enzymes and coenzymes which transfer electrons from each of several of the Krebs cycle intermediates (like succinic or alpha-ketoglutaric acid) through cytochrome(s) and cytochrome oxidase to oxygen. When such oxidation is linked to phosphorylation of compounds like AMP, ADP, and arginine to yield their high energy forms, the phenomenon is referred to as oxidative phosphorylation.

The (sum total of) processes involved appear to be limited more or less to the cell mitochondria, now considered to be organized respiratory particles.

The cytochrome system.—At the risk of being chauvinistic, one can point to the fact that Keilin's original characterization of the cytochromes included their description in 30 species of insects (65). It is also interesting that he found honey bee thoracic muscle the best material for such study of the cytochromes and noted that insect flight muscle had the highest concentrations among the many different (plant and animal) preparations which he examined. Subsequent studies with Hartree (66, 67) lead to a further clarification of the components of the cytochrome system and their biological importance, including the recent identification of cytochrome-*e*, a new component intervening between *b* and *c* [Keilin & Hartree (68)]. Interestingly too, Sanborn & Williams (69) independently identified a cytochrome-*x* in most tissues of larval *P. cecropia* except the heart and intersegmental muscle, which possesses the combined properties of cytochromes-*b* and -*c* plus succinic dehydrogenase and which may be identical with cytochrome-*e*.

Our knowledge of this phase of oxidative metabolism in insects is marked by numerous scattered works in which were demonstrated cytochrome oxidase in the codling moth by Graham (38), in American cockroach femoral muscle by Barron & Tahmisian (39), in orthopteran embryos during late diapause by Allen (70), in *D. melanogaster* larvae by Spirtes (56), and in adults of *O. fasciatus* by Collias and co-workers (57). The part played by the cytochrome system system in energizing metamorphosis has been studied by Wojtczak (71) in the greater wax moth larva, *Galleria mellonella* (Linnaeus); by Ludwig (72) in the Japanese beetle, *Popillia japonica* Newman; and by Sacktor (73) in normal and DDT-resistant strains of house flies. Enzyme activity followed the usual U-shaped oxygen consumption curves in each case, although Sacktor found a cyanide-insensitive (and presumably flavin by-pass) system involved in the respiration of developing pupae of both strains. Sacktor & Bodenstein (74), Harvey & Beck (75), Allen & Richards (76), and McShan, Kramer & Schlegel (77) all confirmed Barron & Tahmisian's report (39) of higher male than female American cockroach leg muscle cytochrome oxidase. Sacktor & Thomas (78) also found higher succino-cytochrome reductase in the male than in the female American cockroach muscle, and Harvey & Beck (75) also identified cytochrome-*a*, -*a*$_3$, -*b*, and -*c* in such muscle homogenates, whereas Allen & Richards (76) also found succinic acid in such muscle homogenates as well as in thoracic muscle homogenates of adult *Sarcophaga bullata* Parker. McShan, Kramer & Schlegel (77) also reported succinoxidase and cytochrome oxidase in thoracic muscle homogenates of *Leucophaea maderae* (Fabricius).

A few attempts at connecting the toxic effects of insecticides with the cytochrome system followed Sacktor's earlier findings of higher activity of cytochrome oxidase in the Ellenville strain of DDT-resistant house flies (79); they consisted of *in vitro* studies by Morrison & Brown (80), Lud-

wig, Barsa & Cali (81), and Tomizawa & Koike (82). Their results are inconclusive as regards the possible action of insecticides or their effects upon normal metabolic pathways in normal or resistant insects.

Williams and his co-workers have contributed immensely to our understanding of the dependence of certain of the enzyme components of the oxidative system upon hormones for their production, in diapausing and metamorphosing *P. cecropia* silkworms. They demonstrated that the synthesis of cytochrome oxidase is related to the endocrine release by the brain, and that synthesis of cytochrome-*c* is dependent upon the function of the prothoracic glands. Particularly significant is their evidence for the vital role of the cytochrome system and inferentially oxidative metabolic pathways in energizing morphogenesis in this species [Williams *et al.* (83 to 88)]. In a parallel study of the possible hormonal control of metamorphosis through the cytochrome system in *Drosophila virilis* Sturtevant, Bodenstein & Sacktor (89) could find no similar interrelationship between hormones from the prothoracic glands or the corpora allata and the production of cytochrome oxidase.

Insect sarcosomes.—Despite the many clues from scattered reports (some mentioned above) indicating the existence of a vertebrate-like oxidative pathway from Krebs cycle intermediates via the cytochrome system to molecular oxygen, the localization of the site of such organized oxidative activity was not technically feasible until the appearance of a very important paper by Watanabe & Williams (51). This established without question that the interfibrillar sarcosomes of insect flight muscles are in reality giant mitochondria (one to four microns in diameter) which, in *P. regina* possess all histochemical, spectroscopic, and enzymatic properties of mammalian liver mitochondria.[7] Such enzymes and coenzymes as cytochromes-*a*, -*b* and -*c*, cytochrome oxidase, glycerophosphate dehydrogenase, malic and pyruvic dehydrogenases, succinoxidase, but no lactic dehydrogenase, were found to be present. Subsequent studies to this initial (*in situ*) localization of biochemical function in the sarcosomes of insect flight muscle included the identification of succinoxidase, $DPNH_2$ oxidase, cytochrome-*c* reductase, and the cytochromes by several workers [Levenbook & Williams (92, 93); Sacktor (58)].[8] The adaptation of visual spectroscopic procedures to a rapid, sensitive spectrophotometric procedure (measuring and recording quantitatively changes in optical density, with reduction of cytochrome and pyridine nucleotide enzymes upon addition of suitable substrates) has permitted Chance (95) to estimate relative and absolute concentrations of components of the cytochrome system in "blow fly" mitochondria. He later (96) identi-

[7] For an excellent summary of the biochemical functions of liver mitochondria the reader is referred to Schneider (90); for some of the most recent ideas on the intricacies and anomalies of oxidative phosphorylation studies with mitochondrial preparations, see Siekevitz & Potter (91).

[8] For a good, succinct review of the status of the biochemistry of insect flight muscle to that date, the reader is referred to Levenbook (94.)

fied a "Slater-like" factor between cytochrome-c and succinic acid [see Slater (97)] and quantitatively confirmed Keilin's (65) early observation of very high cytochrome content in insect flight muscle, as compared to mammalian heart sarcosomes.

Sacktor's research on insect metabolism has contributed as much as any other insect physiologist's to our understanding of oxidative metabolism by insect flight muscles and their mitochondria, especially (44, 58). Particularly important has been his localization of the site of oxidative utilization of citric acid intermediates within the mitochondria, which, together with the sarcoplasm of house fly muscle also are involved in oxidation of all the known intermediary components of the Embden-Meyerhof system, except phosphopyruvate.

Oxidative phosphorylation in insects.—The virtually simultaneous demonstration of oxidative phosphorylation in insects was accomplished by Lewis & Slater (98, 99) in England and by Sacktor (100) in this country, although Sacktor's earlier paper (101) had indicated his conviction of its likely early confirmation, in the light of such evidence as the presence of ATP and necessary enzyme systems for coupling oxidation with phosphorylation. In a most recent paper, Sacktor & Sanborn (102) have examined some of the effects of temperature on the oxidative phosphorylative activities of house fly sarcosomes and found that not only is there an uncoupling of this activity (in terms of reduced P:O ratios) at temperatures above 25°C., but also an accompanying morphological deterioration in the mitochondria themselves.

Of passing interest is the fact that studies on insect sarcosomes appear to have encouraged similar anatomical and biochemical studies on mammalian heart sarcosomes, which resemble those of insects both morphologically and biochemically [Slater (103); Cleland & Slater (104, 105, 106); Slater & Lewis (99, 107, 108); Slater (109); Holton (110)]. Chance's application of differential spectrophotometric techniques mentioned earlier (95) has permitted direct visualization of the activity of the known components in oxidative metabolism of flight muscle as well as of mammalian mitochondria [Chance & Williams (111)]. It is important to note, however, that size, shape, and biochemical activity of mitochondria may be seriously altered or affected by either the methods of separation or the techniques of study of biochemical activity as well [see Sacktor (100); Sacktor & Sanborn (102); Slater & Cleland (105, 106); Slater & Lewis (107, 108); Harman & Feigelson (112); Watanabe & Williams (113)]. The recent description of sarcosomes in the flight muscles of other dipterans, lepidopterans, hymenopterans, and even coleopterans [Sotovalta (114); Chapman (115)] prefaces another chapter in insect physiology which is rich in research promise.

Related Phosphorus Metabolism

The rapidly growing appreciation, especially in the past decade, of the importance of certain organophosphorus compounds as labile storehouses of comparatively large amounts of readily available energy has continued to

attract the active interest of numerous biochemists and physiologists. An extensive summary of the thinking of many outstanding research workers in this field in the form of summaries of two successive symposia in phosphorus metabolism has recently appeared [McElroy & Glass (116, 117)]. The object of this necessarily brief treatment is to discuss some of the known details of certain phases of phosphorus metabolism in insects, which may be dependent upon or otherwise related to carbohydrate metabolism.

Such studies of phosphorus metabolism in insects have been relatively scarce since the early identification of the adenylpyrophosphates and phosphagen in crustacean muscle by Meyerhof & Lohmann (28, 29, 30) and in insects by Baldwin & Needham (33), Schütze (118), Albaum & Kletzkin (34, 35), and Calaby (36). Some scattered papers include the study of changes in different classes of phosphorus compounds in relation to development [see Buck (119)]. A recent, detailed extension by Levenbook (120) of Khouvine & Gregoire's (121, 122) older study of metamorphosing *C. erythrocephala* correlated total carbohydrate and reducing sugar to various phosphorus components, especially the reciprocal relationship between inorganic and "easily hydrolyzable" P. Similar studies have been made by Niemierko on larval *G. mellonella* (123), on developing grasshoppers by Lu & Bodine (124) and in *C. erythrocephala* by Pettersson (125). The last study correlated nicely the reciprocal concentration of phosphagen with that of ATP as shown earlier by Agrell (27, 52 to 55) and muscle development in the metamorphosing pupa. This report is of interest in light of a current report by Winteringham, Bridges & Hellyer (46) that house flies accumulate phosphagen at rest and consume it during flight, while the ATP and ADP components remain relatively unchanged, as in vertebrate muscle metabolism of phosphorus. As regards muscle metabolism per se, observations by Keilin (65), Watanabe & Williams (113), Levenbook & Williams (92, 93, 94), and Sacktor (44) all suggest that the sarcosomes furnish the energy to the actomyosin of insect flight muscle through phosphorylative build-up of compounds like ATP. This is suggested also by Rockstein's reports on acid and alkaline phosphatase and adenosinetriphosphatase activity as a function of maturation and of senescence in honeybees and house flies (126, 127).

This raises the question of activity of enzymes attacking ATP and other organophosphorus compounds. Sacktor (101) encountered a mitochondria-bound Mg-activated adenosinetriphosphatase and adenylate kinase, a soluble extramitochondrial Mg-activated adenosinetriphosphatase which is inhibited by F ions, an extramitochondrial Ca-activated adenosinetriphosphatase in the fibrils and a soluble pyrophosphatase. In confirming some of these data with myosin extracts [see Needham (128)] from wing and leg muscles of two species of locusts (*L. migratoria* and *G. musicus*) and sarcosomes from adult blow flies *Calliphora stygia* (Fabricius) and *Calliphora fallax* Hardy, Gilmour & Calaby also found that actomyosin from wing muscles had more Mg-activated ATP-ase than that from femoral muscles

(129 to 133). On the basis of other findings as well as their own, they propose that the unit of contraction in insect flight muscle be considered the sarcosome plus the fibril, with the Mg-activated apyrase as the more important enzyme quantitatively. Together with Watanabe & Williams' demonstration of insect sarcosome membrane permeability to ATP as well as to a large number of tested glycolysis and oxidative pathway intermediates (113) and Sacktor's strong evidence (44) for active participation by the sarcosomes in glycolysis and oxidative phosphorylation, there appears to be accumulating rapidly data to suggest an hypothesis which will couple chemical processes preceding and directly involved in triggering the contraction process by the development of tension and finally producing fibrillar shortening.

As for other possible biological implications of insect apyrases, Gilmour & Calaby (130) found that locust apyrase also attacked the (phosphorylated hydrolytic deamination product of muscle adenylic acid) inosinetriphosphate and the diphosphate, ITP and IDP. The recent demonstration by Goldberg & Gilmour (134) for similar nonspecificity of rat muscle apyrase, as well as Siekevitz & Potter's interesting recent studies on nucleotide metabolism in rat liver mitochondria (91), all suggest fresh fields for study of such metabolism in insect mitochondria. A number of papers have appeared recently, which are concerned with the possible role of apyrases like adenosinetriphosphatase in development [Maruyama (135); Levenbook (136); Maruyama (137)], with differences among various tissues of one species [Sacktor *et al.* (138)] and in studies of temperature of activation and normal temperature optima of different species [Chin (139); Steinbach (140); Davison & Richards (141)]. Kenney & Richards (142) actually studied different temperature coefficients for flight muscles versus leg muscles of the giant water bug, *Lethocerus americanus* (Leidy). Interestingly, for this species leg muscle Mg-activated adenosinetriphosphatase activity was much higher than wing muscle enzyme; also the leg muscle enzyme appears to be adapted to lower temperatures, its being more adversely affected by high temperatures; conversely they interpret the temperature coefficients obtained as indicating greater ease of cold inactivation of flight muscle at a higher temperature than leg muscle.

In considering the utilization of high-energy compounds arising from carbohydrate intermediary metabolism, the role of ATP in bioluminescence cannot be ignored (nor can justice be done to this fascinating subject in a few brief sentences). It should be mentioned, however, that elaboration of the fundamental processes in which luciferin, luciferase, and oxygen participate, in addition to a bivalent activating ion (Mg or Mn or Co) and ATP has resulted largely from the continued efforts of McElroy and co-workers (143 to 146). Particularly significant here is the demonstration in the firefly, *Photinus pyralis* (Linnaeus), of an active adenosinetriphosphatase and hexokinase. The latter is interpreted as being important in phosphorylating glucose with ATP, which, although present, may not be available for light production [McElroy (147); McElroy & Coulombre (148)].

Miscellaneous phosphorus metabolism.—Although the exact biochemical

role of other "phosphatases" is still not clear, some specific functions in phosphorus metabolism have been suggested for the alkaline and acid phosphomonoesterases by McElroy & Glass (116, 117). Numerous studies by physiologists especially have demonstrated the intimate relationship between such enzymes and a variety of biological phenomena possessing unusually high energy requirements, such as embryogenesis and development (including histolysis and histogenesis) maturation, growth and senescence, and "active transport" of materials against a concentration gradient [Moog (149); Ross & Ely (150)]. Review of the related literature on such studies in insects have appeared in connection with papers of an experimental nature, by Fitzgerald (151), and Rockstein & Herron (152). Bradfield (153) has also presented firm evidence for the participation of alkaline phosphatase in protein (silk) synthesis in the "goat moth," *Cossus cossus* (Linnaeus), the silkworm, *B. mori*, and a species of spider, either in the actual synthesis or by energizing the release of the finished protein from nucleic acid complexes. Denucé (154, 155) has helped to confirm a definite interrelationship between (acid and alkaline) phosphomonoesterases attacking Na glycerophosphate, the pyrophosphatase attacking Na pyrophosphate, and ribonucleic acid of the silk glands of *B. mori*. Lesher (156) has found similar correlated enzyme activity and nucleic acid and polysaccharide distribution in the salivary glands of *Drosophila robusta* Sturtevant. In several papers (some mentioned above) optimal conditions for the study of such enzymes have been worked out and may be useful for studies of either a fundamental or applied nature [Fitzgerald (151); Rockstein & Herron (152); Rockstein (157)]. Such reports show that these enzymes readily fall into categories already established for similar vertebrate and microbial enzymes [see Folley & Kay (158, 159); Bamann & Meisenheimer (160)]. Comparative studies have also been made which suggest species differences, which may (of course) be the result of using for one species conditions of study previously established as being optimal for another [Rockstein & Levine (161); Rockstein & Inashima (162)]. In addition to current studies in the present author's laboratory on the possible role of these enzymes as well as of Mg activated adenosinetriphosphatase in DDT-resistance of house flies, Tomizawa & Koike (82) have also studied the effect of several insecticides on alkaline phosphatase (and fumaric and citric dehydrogenases) in the rice stem borer, *Chilo simplex* Butler.

Concluding Remarks

To be sure, conclusive evidence for the existence of anaerobic and aerobic mechanisms, virtually identical with those found in higher vertebrates and microorganisms in the metabolism of carbohydrates, is limited chiefly to the house fly and American cockroach. Nevertheless, data of a scattered and fragmentary nature suggest the existence of the Embden-Meyerhof system (with such by-passes as the hexosemonophosphate shunt), the Krebs citric acid cycle, and mechanisms for oxidative phosphorylation in other species as well.[9] We can now expect continued intensification of the search for these

and alternative pathways in a variety of insects with different ecological preferenda and different degrees of motor ability and efficiency. With the realization by nonentomologists of the advantages of studying fundamental biochemical processes of this kind in insect flight sarcosomes (as relatively huge, readily obtainable and readily separable cell entities in which oxidative activity is organized), we can also anticipate the continued increase in participation by biochemists and general physiologists in this area of insect physiology.

This optimistic note should not be sounded without the addition of one of caution. The conclusive demonstration of the existence of a particular metabolic pathway requires evidence for the existence of (a) the enzyme system, including necessary coenzymes and activators, (b) the substrates involved, and (c) the expected intermediary metabolites and final products of such a metabolic system or cycle. Strict attention is necessary to details of incubation temperature, time and pH, concentrations of enzyme substrate, the inclusion of necessary ions and, finally, the appropriateness of the extracting medium. In mitochondrial studies the last is especially important, because of the possible variations in morphological as well as biochemical properties under different conditions of preparation [Sacktor (100); Sacktor & Sanborn (102); Watanabe & Williams (113); Slater and co-workers (105 to 108); Harman & Feigelson (112)]. Indeed, in a most recent paper, Ludwig & Barsa have reported an anomalous decrease in respiration by Japanese beetle eggs and by *T. molitor* eggs or larvae, as a result of homogenization under refrigerated conditions (164). The time factor above all is most critical both from the standpoint of the kinetics of enzyme study (and the need to maintain a zero order reaction) and the possibility that some of the activity measured is really exogenous, i.e., is attributable to contamination. For example, Terriere (165) has found a drastic curtailment in what he had thought was endogenous respiration by house fly preparations when he controlled bacterial contamination.[10]

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[9] The ability of acetone powder extracts of cell-free homogenates of the pea aphid, *Macrosiphum pisi* (Harris) to oxidize a number of components of the glycolytic and Krebs cycles clearly suggests the presence of such cycles in this insect [Newburgh & Cheldelin (163)]. Interestingly too, they demonstrated the formation of pentose, sedoheptulose, triose and hexose compounds, which also indicates the possible intervention of the hexosemonophosphate shunt within the glycolytic system of this species, as Chefurka (47) so demonstrated for thoracic muscles of the house fly.

[10] In none of the papers which he has consulted on insect intermediary metabolism, does the present author recall having seen indicated the possible elimination of this extraneous factor of contamination from enzyme incubation mixtures. Although toluene has been so employed in classical enzyme studies, it is said to have occasional denaturing effects on enzymes. Although this is not important except for incubation periods of one hour or more, the author has always employed a drop or two of chloroform in each incubation mixture for enzyme studies he has made, as a matter of customary procedure.

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THE PHYSIOLOGY OF INSECT CUTICLE[1]

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The physiology of the insect cuticle was the subject of a detailed survey by the present author in 1948 (68) and a complete monograph by Richards in 1952 (58). The present review will deal with developments in the past eight years; the previous article (68) will be used as the starting point and will be quoted as the source of references to the earlier literature. Almost all the conclusions that were reached eight years ago have since been questioned. Many have been substantiated, others will require revision, and on many more the last word has not yet been heard.

THE COMPOSITION OF THE SOFT CUTICLE

It is generally agreed that chitin as isolated from the cuticle is a poly-acetylglucosamine. When purified chitin from lobster cuticle or from fly puparia is broken down by chitinase from the gut of the snail, *Helix aspersa* Linnaeus, it gives rise to N-acetyl-D-glucosamine together with a trace of D-glucosamine (20). But as Rudall (62) points out the nitrogen content of purified chitin is usually about 6.4 to 6.5 per cent, whereas the theoretical value is 6.9 per cent. This may indicate the presence of non-nitrogenous impurities, the substitution of —OH groups or the presence of a few non-amino sugars. From studies of the infrared absorption spectra of chitin and chitosan, in conjunction with x-ray diffraction data, Darmon & Rudall (7) conclude that in the crystalline regions there is a large amount of direct linkage between one acetyl-hexosamine chain and the next. The most important type of linkage appears to be hydrogen bonding between $CO \cdot NH$ groups of adjacent amino-acetyl side chains. Other acetyl groups may perhaps be joined by bonds of the type CO----HO. And during chitosan formation, as a result of progressive deacetylation, the principal interchain bonding is of the type OH----OH, as in cellulose.

X-ray analysis of insect cuticle shows that the pure chitin lattice as defined by Meyer and Pankow (68) is very stable, whereas soft insect cuticles show a modified chitin lattice which is highly unstable (61); it changes rapidly to the pure chitin lattice when the protein content is altered by heat or by dilute acids or alkalies. On the other hand, the modified lattice becomes highly stable after fixation of the protein by tanning (p. 39). Rudall therefore concludes that the pure chitin lattice is to be regarded as an end product of degenerative processes; whereas the modified lattice of the natural membranes is attributable to the interaction between protein and polysaccharide. It is perhaps this same association which will account for the

[1] The survey of the literature pertaining to this review was completed in March, 1956.

stability of insect cuticular protein when it is within the cuticle and its high water solubility and low viscosity when extracted.

The extractable protein of the cuticle was termed "arthropodin" by Fraenkel and Rudall and its amino acid composition was described by Trim (68). Further detailed study by Hackman (17) has confirmed these conclusions in showing that preparations from seven species of insects from three different orders were uniformly devoid of sulphur, phosphorus and carbohydrate, and all contained the same series of amino acids. But it is unlikely that "arthropodin" is a homogeneous chemical entity. Electrophoresis shows that it is heterogeneous: in the beetle *Diaphonia dorsalis* Donovan five protein fractions were recognized (22); but the general character of this protein mixture seems to be very constant throughout the arthropods (33).

There has been much discussion on the nature of the association between "arthropodin" and chitin. Hackman (21) has shown that at alkaline pH N-acetyl-D-glucosamine is definitely bound by the water soluble protein isolated from larvae of *Diaphonia*. Tyrosine groups, which are plentiful in arthropodin, and free amino groups (notably terminal amino groups provided by glutamic acid, and the ε-amino group of lysine) seem to be chiefly responsible. The type of association is believed to be of the same nature as that between amino acids or peptides and sugars; that is, they form compounds of the Schiff base or azomethine type, which may assume the isomeric N-glycoside form. Such compounds are unstable, being split even in the biological pH range. When Hackman (22) extended his study to the adsorption of the various protein fractions isolated from *Diaphonia* by purified chitin powder from lobster cuticle, he found that those fractions richest in tyrosine residues were preferentially absorbed; there seems little doubt that these residues play an important part in this adsorption. The attachment is quite weak; most of the protein can be readily eluted; and since no adsorption occurs at pH 9 he concluded that neither hydrogen bonds nor covalent bonds are involved in the adsorption.

The general conclusion drawn by Hackman (22) from this work is that there is only a weak bonding between the chitin and the water soluble protein in the soft cuticle of insects [cf. Richards (58)]. But it is well to recall that the polysaccharide chemists (Haworth, Stacey) consistently regard the soft cuticle of arthropods as a mucopolysaccharide or mixture of mucopolysaccharides (68). In any case there can be little doubt that the soft cuticle may vary in composition in different parts of the same insect. For example, I have found that in *Rhodnius* the endocuticle over the greater part of the body gives a negative periodic acid-Schiff reaction [though this becomes positive when the cuticle is being digested by the moulting fluid (p. 51)]; on the other hand this reaction is strongly positive in the flexible membrane of the neck and in the conjunctivae of the limbs (70)

Hardening and Darkening of the Cuticle

An association of protein with polyacetylglucosamine in the soft cuticle may be regarded as established. It was suggested by Fraenkel and Rudall

(68) that when the chitin micelles become orientated under tension, in the cuticle of the *Sarcophaga* larva prior to puparium formation, the associated protein is likewise orientated. In the bristles of the polychaete *Aphrodite* there is similar evidence from the study of birefringence before and after extraction of the protein, that both the polyacetylglucosamine and the hardened protein are orientated (56). Indeed Kroon, Veerkamp & Loeven (44), after studying the changes in the x-ray pattern of the butterfly's wing during expansion and stiffening, conclude that this orientation and close packing of micelles is an essential factor in hardening, as it is in the hardening of silk and cellulose when stretched.

But there is no doubt that chemical stabilization of the protein also occurs. The current interpretation of the horny substance in the cuticle derives from Pryor's observations on the oötheca of the cockroach (68). This is formed from the interaction of the secretory products of the two colleterial glands: the left gland secretes a watery solution of protein and a polyphenol-oxidase, the right gland was believed to secrete protocatechuic acid, which is oxidized to quinone when the two secretions are mixed, and the protein is thus tanned to produce "sclerotin."

The process has been reinvestigated in *Periplaneta americana* (Linnaeus) by Brunet & Kent (6). They have found that in fact the protein, polyphenol oxidase, and protocatechuic acid are all products of the left colleterial gland. But the diphenol is combined in the form of the 4-*o*-β-glucoside of proto-catechuic acid. The right colleterial gland secretes a β-glucosidase. Thus when the two secretions are mixed the β-glucosidase liberates the protocatechuic acid from its glucoside, and the oxidative formation of quinone and the tanning of the protein can then proceed. Brunet & Kent were able to separate these various components by paper chromatography. Pryor had been misled into locating the diphenol in the right gland because in *Blatta orientalis* Linnaeus this gland gives a positive argentaffin reaction attributable to some unknown cause.

Pryor extended his theory of sclerotin formation to the insect cuticle, and evidence has continued to accumulate in support of this interpretation. Of thirteen species of insects examined by Hackman, Pryor & Todd (23), ten contain 3,4-dihydroxyphenyl acetic acid; in seven species this is the only diphenol detected; in two species it is accompanied by 3,4-dihydroxybenzoic acid, and in one species by 3,4-dihydroxypropionic acid; in two species 3,4-dihydroxyphenylalanine has been isolated in addition. It will be interesting to see whether other diphenols exist in masked form as glucosides (6). In the combination of orthoquinones with amino acids or proteins the linkage does not occur between the quinone groups and the amino groups as has been commonly supposed, but the nitrogen of the amino group becomes directly attached to the quinone nucleus, the quinone being simultaneously reduced to the diphenol (23, 24).

The organic chemistry of diphenols and quinones is fully reviewed by Mason (48). Quinones react with amino, imino, sulphydryl, and free hetero-cyclic groups, and since proteins contain plenty of such groups they will

react readily with quinones. In the elytra of the beetle, *Aphodius howitti* Hope, as studied by Hackman (18), there was 55 per cent of chitin, the remainder being fully hardened protein (sclerotin). In this material no free amino groups remain. This strongly suggests that these have reacted with quinones. The available free amines in arthropodin are the N-terminal amino groups, which are plentiful in the water soluble proteins of the cuticle, and the ε-amino group of the lysine present. It is interesting that when the sclerotin was hydrolysed, although most of the amino acid residues were the same as those in the soluble proteins of the soft cuticle, lysine was absent (18).

According to the interpretation put forward by Mason (48), the N-terminal amino groups of protein are the first to react, forming mono-substituted nuclear derivatives. The product is a colourless aminohydroquinone. But in the presence of excess quinone such substances are oxidized to the corresponding quinones, and provided a second substitutable position is available these may react with a second N-terminal amino group to form disubstituted nuclear derivatives. This, however, is possible only when the reacting quinone is derived from protocatechuic acid, in which the carboxyl group is readily displaced by amines. End to end linkage of arthropodin molecules in this manner is therefore limited by the supply of protocatechuic acid. The o-quinones derived from 3,4-dihydroxyphenyl lactic acid and 3,4-dihydroxyphenyl acetic acid can form only mono substituted products. After all the N-terminal groups have been bound to quinone nuclei, the ε-amino groups of the lysine residues react to form mono substituted derivatives. Mason points out that the absorption spectra observed by Hackman (19) during the interaction of arthropodin with o-benzoquinone contain a maximum at 480 mμ during the early phase. This maximum corresponds with that of amino acid derivatives of o-benzoquinone and confirms the mechanism suggested above for the initial steps in sclerotization.

The final steps in hardening yield products characterized by general absorption in the visible range of the spectrum. The physical properties of these products suggest that cross-linked polymerization has occurred (48). One mechanism, proposed by Hackman & Todd (24), after the primary condensation of the free amino groups with o-quinones, was indole formation followed by oxidative polymerization. In view of the high ratio of pigment to protein observed in sclerotized cuticle, Mason (48) considers that the primary reactions with quinones probably bind all reactive sites on the arthropodin molecules. "The final stages of tanning must therefore involve polymerization and copolymerization of the tanning quinones with arthropodin itself or with an arthropodin quinone conjugate. Secondary cross-linkages should form readily through electrostatic forces involving carboxylate, or by hydrogen bonding of the type: quinone carbonyl to peptide amide." Any unacetylated amino groups in the N-acetyl-glucosamine chains would likewise react rapidly with quinones. It is possible, as Mason (48) suggests, that

this is one source of the primary valence linkages between chitin and sclerotized proteins.

Being unable to demonstrate any alcohol-soluble quinone precursor from the byssus and periostracum of *Mytilus edule* Linnaeus, Brown (5) suggested that the phenolic hydroxyl groups of the tyrosine residues in the protein might be oxidized *in situ* to produce quinones, so that the protein might then tan itself. This idea has been taken up by Blower (4) in Myriapoda, by Hackman (19) and by Dennell & Malek (9 to 13) in insects. The tyrosine content of arthropodin is fairly high, and it has been shown that tyrosyl residues can be oxidized enzymically even when the amino and carboxyl groups are chemically combined (66), but as Mason (48) writes, "before this mechanism can be accepted, the oxidation of homogeneous arthropodin fractions by homogeneous arthropod phenolases must be demonstrated, and the rate of reaction of this system must be compared with the rate of sclerotization due to quinone tanning."

The foregoing account represents the general provisional picture arrived at by the organic chemist. How complex, and how obscure at the present time, is the process of sclerotization as it occurs in the insect cuticle is made evident by a series of papers by Dennell & Malek (8 to 13) on the cuticle of the cockroach *Periplaneta americana*. The exocuticle in the sclerites of *Periplaneta* consists of an outer zone which is amber or brown in colour, and an inner zone, which resembles the outer in being homogeneous and refractile but is yet colourless. [This layer is termed "mesocuticle" by Schatz (63).] These layers are impregnated with a protein and with cholesterol, the quantities of these impregnating substances being much greater in the outer zone than in the inner. The protein is believed to be tanned in part by quinones produced from diffusible dihydroxyphenols; it is also suggested that (as described above) quinone may be produced from tyrosine residues forming part of the protein chains so that the protein may be described as self-tanning. Finally, the sterols are thought to play some undefined part in the hardening process. In short these authors regard the material which impregnates the exocuticle in *Periplaneta* as being of essentially the same character as the "cuticulin" or tanned lipoprotein of the inner part of the epicuticle (p. 43) [cf. Pfaff (54)].

The arguments brought forward in support of these ideas are of a general nature, largely histological and histochemical. Clear-cut chemical evidence is still lacking. Indeed the whole problem of sclerotization of the cuticle is far from being fully solved. Tanning by quinones seems always to lead to darkening; but, as Brunet & Kent (6) point out, such structures as the lateral pronotal extensions in *Blaberus* are parchment coloured, more or less transparent, and yet of considerable hardness [cf. Schatz (63)]. The process of sclerotization may prove to be of more than one kind.

In the cuticular proteins of insects sulphur appears to be absent; but in the exocuticle of the scorpion *Palamneus swammerdami* disulphide bond-

ing is present in addition to tanning (40); and in the epicuticle phenolic tanning is said to be absent (41), the bonds being broken by treatment with alkaline sodium sulphide in much the same way as cuticle hardened by phenolic tanning is disrupted by diaphanol (42).

The hardness of sclerotized cuticle varies in different regions according to physiological needs. The hardest parts, such as the mandibles of chewing insects, were tested by Bailey (1) using scratch tests according to Mohs' scale of mineral hardness. They were found to approximate to "3" on this scale, the specimen of calcite being scratched with difficulty. This is harder than tin, copper, zinc, or silver. Thus it is of interest to note that the calcium carbonate present in the cuticle of Crustacea in the form of calcite does not increase the hardness of the cuticle; indeed, as Pryor pointed out, these animals have a sclerotin surface to their hardest cuticular structures.

Enzymes and precursors in the hardening process.—A complete understanding of the sclerotization and darkening of the insect cuticle must include a knowledge of the precursor substances and the enzymes involved. Malek (47) has confirmed on the cockroach Dennell's earlier observation on *Sarcophaga* (68) that the Nadi reaction for oxidizing enzymes appears first in the epidermis and later becomes concentrated in the epicuticle. From this observation, and the histological changes in the cuticle, it is concluded that polyphenol oxidase in the epicuticle is responsible for quinone formation, which leads to the progressive sclerotization of the cuticle inwardly. But, as Mason (48) points out proof of this conception will demand "isolation, purification and characterization of the enzyme, and its localization in arthropodal integument by techniques of greater reliability than those utilizing only the Nadi reagent."

The precursor substances must come from the blood. There is little doubt that the tyrosine-tyrosinase system in the blood and tissues is important in relation to the cuticle; and this leads on to the whole subject of melanin formation, which it is impossible to include in this review. A few recent observations only will be recorded.

Ohnishi (50a) noted that in the third instar larva of *Drosophila*, tyrosinase activity increases rapidly shortly before puparium formation, reaches a maximum at the time of initiation of pigmentation and hardening, and then falls again. This is in agreement with observations on other insects (68). But the oxidase activity in the cuticle of the pupating larva of *Drosophila* is said to be different from that of typical tyrosinase or polyphenol oxidase, and it is not cytochrome oxidase (51). On the other hand, during pigmentation in the adult *Drosophila*, there is no rise either in tyrosinase activity or in the free tyrosine content of the blood. Ohnishi (52) therefore suggests that the mechanism of imaginal pigmentation may be quite different from that of puparium pigmentation. In the body fluid of the silkworm, as shown by Ito (29), phenol oxidase likewise increases towards pupation, reaching a maximum several hours after pupation has occurred. It becomes distributed

throughout the cuticle, but varies in amount in different parts; in tyrosine solutions the exocuticle darkens but the endocuticle does not.

In the blood of *Gastrophilus* larvae Levenbrook (46) found a phenolic substance which increases in concentration towards pupation. This is not tyrosine, or 2,5 or 3,4-dihydroxyphenylalanine ("dopa"), but has the absorption spectrum of an aromatic compound. The nature of this substance is unknown, but since it is oxidized in the presence of *Gastrophilus* tyrosinase, it may well be of importance in sclerotization. And yet another possibility has been advanced by Pryor (57). Working with extracts from mature larvae of *Calliphora* and *Lucilia*, he demonstrated the presence of small amounts of "dopa" and considerable quantities of kynurenine and 3-oxykynurenine. He suggests that instead of dihydroxyphenols derived from tyrosine being used to tan the cuticle, there may be an alternative method in which aminophenols derived from tryptophan are used.

THE EPICUTICLE

The account of the epicuticle as set out in the earlier review (68) was based on the study of the stages in which this layer is formed. During this process a series of strata of differing composition are deposited one upon another. This led to the description of the epicuticle as a laminated structure, although in the fully hardened cuticle it is often impossible to get any clear evidence of these separate strata.

The layers in question were said to be four in number: (*a*) The "cuticulin layer," the epicuticle as seen with the microscope, which is thought to be composed of lipoprotein subsequently tanned by quinones; (*b*) A soft or semifluid layer which reduces ammoniacal silver hydroxide very actively. Since the commonest materials in living tissues which have this property are diphenols, this was called the "polyphenol layer." The existence of this layer during the deposition of the epicuticle in *Rhodnius, Tenebrio* etc. is not in question; for the substance can be readily displaced mechanically before exposure to ammoniacal silver, and areas of cuticulin free from this silver reducing layer can then be seen. But it is very doubtful whether anything in the nature of a layer persists in the fully hardened cuticle. (*c*) A layer of more or less crystalline wax then appears over the surface of the cuticle. This is detectable by its waterproofing and hydrophobe properties, both of which are removed at once by cold chloroform. (*d*) Finally the "cement layer" [called tectocuticle by Richards (58)] is poured out from the dermal glands to cover the wax.

Advances during the past eight years have revealed various departures from this scheme and have emphasized that this stratified structure represents merely a description of the process of deposition and is not to be regarded as a true picture of the fully hardened epicuticle. Thus Kramer & Wigglesworth (39) point out that wax can pass through the unbroken cuticle over the wax secreting epithelium of the honey bee, or through the sclero-

tized cuticle and cement layer of the cockroach. They therefore suggest that the laminar picture of the epicuticle may be too schematic and that the layers may be impregnated to a varying extent by the diffusing waxes.

The epicuticle as visible in histological preparations of the blowfly larva (*Calliphora*) and the cockroach (*Periplaneta*) is believed by Dennell & Malek (9 to 13) to consist of two layers: an inner "cuticulin" layer of tanned lipoprotein, the lipoid being of sterol type, and an outer layer, even more resistant to solvents, in which the lipoid is believed (on the evidence of x-ray diffraction) to be of paraffin chain type. Both these layers give a positive Nadi reaction for oxidase at the time of moulting. In the cockroach, as in other insects, the epicuticle contains no chitin. But in the scorpion *Palamneus* and in the centipede *Scolopendra* the epicuticle was found by Krishnan and others (43) to contain both lipoprotein and chitin. The chitin was demonstrated by estimating the glucosamine formed after hydrolysis in a sealed tube and by the x-ray pattern after treatment with potassium hydroxide. The chitosan reaction was negative.

The epicuticle covering the sense plates and adjacent cuticle of the antenna in the honey bee, as studied by Richards (59), likewise consists of two layers: an inner amber coloured protein epicuticle or cuticulin layer, and an outer layer which stains with haematoxylin, osmium tetroxide, and ammoniacal silver nitrate. Richards believes that this layer presumably corresponds with the wax plus "polyphenol" layer of *Rhodnius* etc. or with the "lipoid epicuticle" of Dennell & Malek. Dermal glands are wanting in the honey bee, and there is no cement layer or tectocuticle. In the adult silkworm, also, the epicuticle is double: the outer layer stains with Heidenhain's haematoxylin, osmic acid, and ammoniacal silver hydroxide, suggesting the presence of both lipide and phenolic substances; the inner layer is not stained (30).

The deposition of the epicuticle has been followed in two insects: in the caterpillar of *Diataraxia oleracea* (Linnaeus) by Way (67) and in the larva, puparium, pupa, and adult of *Calliphora erythrocephala* (Meigen) by Wolfe (71, 72, 73). In *Diataraxia* the process agrees closely with that described in *Rhodnius* and *Tenebrio* (68), except that no "polyphenol layer" is discharged upon the surface of the cuticle. But in the larva of *Calliphora* the process is quite different. Only the two layers [which were recognized by Dennell (68) in the larva of *Sarcophaga*] are present in the epicuticle. The outer epicuticle is formed from droplets on the cell surface, which coalesce to form a continuous lipoprotein layer. Then the cytoplasmic processes, which will later become the pore canals, add beneath this more protein and lipoid (cuticulin) to form the inner epicuticle. Surface layers of orientated wax and cement are not formed, and there are no dermal glands. A positive Nadi reaction develops in the inner epicuticle but is limited to the tips of the cuticular spines. In the larva of *Hypoderma bovis* (Linnaeus), on the other hand, Enigk & Pfaff (15) record the presence of a thick wax layer.

Cement layer.—In the larva of *Diataraxia* the cement layer is probably

not more than 0.1μ thick. In some places it forms a continuous sheet, which covers the tubercles on the cuticle of this caterpillar, but for the most part the tips of these tubercles project through it. It may thus act as a supporting framework to the wax layer, but it does not form a complete protection for it. The cement is produced by Verson's glands. The secretion gives a positive ninhydrin reaction and contains some lipoid, staining with Sudan Black B, perhaps discharged in the form of lipoprotein. The plug which forms in the duct of the gland appears to contain phenolic materials; perhaps it consists of tanned protein. The intercalary cell of Verson's glands stains with ammoniacal silver hydroxide and is perhaps the source of the phenolic material concerned. On this evidence Way suggests that the cement layer may consist of lightly tanned lipoprotein (67; cf. 68).

Likewise in the cockroach, *Periplaneta* (39), the material which forms the cement layer is poured out from the dermal glands before or shortly after moulting and fills the shallow depressions in the cuticle. If treated very mildly with chloroform, when first deposited, it gives a granular blackening with ammoniacal silver hydroxide and was, therefore, supposed to be tanned lipoprotein material, as in *Tenebrio* and other insects. But a reinvestigation of the extractable lipides in the cockroach cuticle by Beament (3) has led to a different conclusion.

There is evidence that the lipide components of the natural grease of the cockroach are fully saturated. But Beament noted that the grease is a strong reducing agent. The component responsible closely resembles shellac in its solubility and other properties. It is strongly acid, has an iodine number of 12 (a sample of shellac from *Laccifer lacca* Kerr had an iodine number of 10), and suitably treated it gives rise to a fine white precipitate which closely resembles the dihydroxycarboxylic acid in shellac. Beament suggests that this shellac-like material represents the "cement layer" of the cockroach cuticle. The reaction of the carbohydrate portion of lac with ammoniacal silver hydroxide is so similar to the reaction of polyphenols as to suggest that in some instances it may prove to be the cuticular tanning material, and that what one insect, the lac insect, produces in quantity, others produce only in the minute amounts necessary to participate in the polymerization with proteins and waxes in the hardening of the cuticle (3).

The cement which covers the puparium of *Phormia regina* (Meigen), or secures the puparium of *Drosophila* to its surroundings, is the product of the salivary glands. It is a proteinaceous material containing glucosamine. The nature of the hardening process is unknown (16, 50).

WAX SECRETION AND WATERPROOFING OF THE CUTICLE

Wax secretion.—In a series of closely related saturniid moths there seems always to be an inverse relation between the amount and the melting point of the lipides both in the cocoon and in the epicuticle: the greater the amount of lipide the lower is the melting point (37). But besides these specific differences there may be adaptive changes in a given species. When the

larvae of the rice stem borer, *Chilo simplex* Butler, hibernates in dry straw, the cuticular wax is larger in amount than when hibernating in the moist stubble of the paddy field. It increases during hibernation in both groups of larvae so that the rate of transpiration falls, particularly in the "dry" larva (35, 49). In *Pieris brassicae* (Linnaeus) larvae there is an inverse relation between atmospheric humidity and the amount and melting point of the cuticular wax (38). In the moth, *Dictyoploca japonica* Butler, the epicuticular lipide is larger in amount and has a higher melting point when reared at high temperature: after warm treatment the pupa contained some 29.1 mg. of lipide with a melting point of 60.3°C.; after cold treatment, some 12.0 mg. with a melting point of 39.9°C. (36, 49).

The way in which the long chain waxes of the cuticle are secreted and transported through the cuticular membranes is still an unsolved problem, but important observations have been made in this field. In the newly moulted pupa of *Tenebrio* the cuticle is smooth and glossy and is covered with a "primary wax layer" of perhaps 0.15μ in thickness. Within 4 to 12 hr. after moulting the cement layer is poured out over this, and between that time and 30 hr. the surface of the cuticle acquires a dull matt appearance. This is a result of the separation of a surface wax layer in the form of fine filaments. If smoothed out it would form a layer about 0.75μ thick; it shows the same molecular and crystalline structure as the primary wax layer (27).

Holdgate & Seal (27) interpret these observations as follows. We have seen that the cement in *Periplaneta* is composed of shellac-like material (3). Shellac contains wax as a main constituent, and in *Laccifer lacca* itself the wax may be present in excess and form a surface bloom. If *Tenebrio* pupal cement were similar to shellac, the appearance of the surface wax filaments might be attributable to the crystallization of excess waxes on the surface of the cement during its hardening. This hypothesis is attractive since it is otherwise necessary to postulate the secretion of the surface wax filaments through the primary wax and cement layers. The authors elaborate the view that cuticular wax permeates the cement layer (39) and suggest that we may be dealing not so much with a laminated structure as with a single "outer epicuticle," whose main component is wax, but which contains a central zone of bound carbohydrate (shellac) giving strength and resistance to solvents (27).

Somewhat similar views were put forward by Wolfe in a study of the cuticle of *Calliphora*. It appears that waxy substances are liberated on to the surface of the puparium during the process of hardening and darkening (73). The pupal moulting fluid of *Calliphora* contains a protein-lipoid substance, and when the moulting fluid is absorbed, it leaves behind on the surface of the epicuticle of the adult fly a hydrophobe protein-lipoid film, which is probably important in restricting water loss immediately after emergence. But during the hardening of the cuticle, waxy materials appear on the surface; perhaps they are liberated from the cuticle during sclerotization. It may be recalled that there is no cement layer in these flies; the wax is

freely exposed on the surface (72). It may be that this is correlated with the presence of microtrichia and that these take the place of a cement layer as a protection for the wax.

In all the insects so far considered in this section, the wax is pictured as being solubilized by association with protein or other substances. Quite a different mechanism of wax transport has been demonstrated in the cockroach by Beament (2, 3). The grease freshly extracted from the cuticle of *Periplaneta* has a melting point of about 34°C. When exposed to the air for some months it gradually hardens to a wax melting sharply at about 55°C. This change does not occur if the grease is sealed in a tube, even when exposed to oxygen in the light; on the other hand, hardening can be brought about within one hour if the grease is heated in air or *in vacuo* at 100°C. In fact the grease consists of a hard wax dissolved in a volatile solvent. Among the series of paraffins and alcohols of which the wax is composed, there are compounds with a chain length in the range C_8 to C_{12} which provide the solvent. The average molecular weight of the solvent lies between 120 to 170, and of the hard wax 300 to 350, but it is unlikely that there is any sharp dividing line between "wax" and "solvent." Of a large range of wax solvents tested, only mixtures of octane and octyl alcohol or decane and decyl alcohol are miscible in all proportions with insect waxes and form synthetic greases with beeswax just like the natural grease of the cockroach.

It has been shown in the past (68) that films of insect wax, or the intact insect cuticle, become permeable to water if exposed to the vapour of such wax solvents as chloroform. On the other hand, the vapour of an octane-alcohol mixture is almost unique among organic wax solvents in causing no change in the permeability of the natural cuticle to water, and exposure of an artificially waxed membrane to this vapour and to water actually enhances its impermeability. Beament suggests that these solvents may assist in the spontaneous separation of the waxes from the "polyphenol layer" and that they may account for the passage of waxes through the hardened cuticle during repair processes (3). It remains to be proved whether similar solvents are present in other insects.

Waterproofing of the cuticle.—There has been further confirmation of the importance of the wax in the waterproofing of the cuticle. Hurst (28) has shown that the electron diffraction pattern of the lipides on the epicuticle of the *Calliphora* pupa is similar to that of artificial collodion-paraffin or collodion-fatty acid membranes. He concludes that the cuticular waxes consist of a three dimensional arrangement of orthorhombic microcrystals, orientated perpendicular to the cuticle surface. Exposed to high beam currents the artificial collodion-fatty acid membrances and the pupal skin undergo irreversible disorientation. There is a transition from an orthorhombic to a hexagonal type of lateral close packing and an irreversible increase in permeability to water. On the other hand, if these thermo-labile lipides are removed from the pupal skin with chloroform, the residual or bound lipides of the cuticulin layer give a diffraction pattern that does not fade on heating,

suggesting that the orientation of the crystals is maintained by intimate association with the structural proteins of this layer.

It is well established that it is the superficial wax layer which is most important in waterproofing. In the pupa of *Tenebrio*, if the surface is progressively abraded by rolling in a glass vessel with activated charcoal for an increasing number of times, there is a progressive increase in water transpiration (27). It is claimed by Glynne Jones (34) that in the worker honey bee, in which, as we have seen, there is no cement layer over the wax, activated charcoal will disrupt the wax layer by adsorption and without abrasion. But the point is difficult to prove, for even if the dust is suspended in water and allowed to dry on the cuticle, very large surface forces are generated which might well exert a mechanical effect on the cuticular wax.

We have seen that "dry" hibernating larvae of *Chilo* are more impermeable than larvae hibernating in "wet" conditions. The "critical temperature" of transpiration is likewise greater in the "dry" larvae: 45°C. for the "dry" larvae, 35°C. for the "wet"; and correspondingly the melting point is higher in the wax from the dry larvae (35). Striking changes in the permeability of an individual insect are to be seen also at the time of moulting (27). The newly moulted pupa of *Tenebrio* has an apparent "critical temperature" of about 38°C.; the mature pupa an apparent critical temperature of about 50°C. But this change is not a result of the addition of the surface wax layer (for it is unaffected if this is removed with chloroform); it is attributable to the cement. Indeed it is shown by Holdgate & Seal (27) that in *Rhodnius* also, whereas the apparent critical temperature 30 hr. after moulting is about 55°C., the value within 30 min. after moulting, is little more than 35°C. We have seen that the progressive increase in impermeability of cockroach grease is a result of the evaporation of volatile components (3). But that is not so in *Tenebrio* or in *Rhodnius;* the improved waterproofing is attributable to the cement.

The wax extracted from the pupa of *Tenebrio*, examined by transmission electron diffraction, gives a ring pattern typical of an orthorhombic straight chain hydrocarbon compound ($a = 7.36$ Å; $b = 4.89$ Å; C–C distance in the hydrocarbon chain 2.5 Å). It was suggested by Beament (68) that the more or less abrupt increase in permeability in the waterproofing film of insect wax, which takes place well below the melting point, might be attributable to a "transition" of the type originally described by Müller in pure paraffins. But Holdgate & Seal (27) followed the a and b unit cell dimensions in *Tenebrio* wax by electron diffraction, from room temperature until complete fusion of the wax had occurred, and there was not the slightest change in the observed spacings.

This led these authors to reinvestigate the nature of the "critical temperature." They recall that an exponential relation between temperature and permeability is well recognized in the diffusion of a fluid through a sheet of solid material. After making certain approximations they arrive at the expression $m = Ze^{-Y/T}$ where m is the mass of water diffusing, Z and Y are

constants, T is the absolute temperature, and e is the base of natural logarithms. If this relation held strictly for the transpiration of water through the insect cuticle, a straight line should be obtained when $\log_{10} m$ is plotted against $1/T$.

In permeable aquatic insects or in *Tenebrio* larvae in which the surface of the cuticle has been slightly abraded, this relation holds, and a straight line is obtained. On the other hand, in insects with a complete layer of waterproof wax, such as *Tenebrio* pupae or *Rhodnius*, the points on the logarithmic plot depart significantly from linearity. Without further evidence it is not possible to explain this result. The most likely explanation is that physical changes in the epicuticular waxes, such as the melting of the softer components, will give rise to an increased permeability over a certain range of temperatures.

It was unfortunate that in the original curves of transpiration published by Ramsay, by Wigglesworth, and by others, the rate of loss of water was plotted directly against the temperature, and the steeply rising drying power of the air was not taken into account. As was indicated by R. W. Salt (1948, in personal correspondence), by Edney (14), and by Shaw (65), this is only too apt to give the impression of a more or less abrupt increase in permeability where none exists. Moreover, as Holdgate & Seal (27) point out, it is only over the "critical region" itself, where the tangent is approximately 45°, that the eye can assess the change in steepness of the curve; by changing the scale on the ordinate the apparent position of the critical temperature can be varied within wide limits. But, provided these limitations are borne in mind, curves of this type, obtained under standard conditions, still afford the most satisfactory way of comparing the permeability of the cuticle in a series of insects. The physical interpretation of the results is another matter and is still to be found.

The waterproofing of aquatic insects has been little studied in the past. Shaw (65) examined the cuticle of the larvae of *Sialis lutaria* (Fabricius). A wax layer of the same order of thickness (0.1μ) as in terrestrial insects is present, but the cuticle is much more permeable to water. There is no sudden change in the rate of transpiration as the temperature rises, no increase in the value of the Q_{10} of evaporation, such as would occur if there were a definite "critical temperature." Similar results were obtained by Holdgate (26) in a series of aquatic insects; when the logarithm of the rate of water loss was plotted against the absolute temperature, a straight line was obtained. A wax layer is shown to be present, but perhaps it is porous or incomplete. For we have seen that these same results are given by highly impermeable insects, such as *Tenebrio*, after abrasion of the cuticle. A possible hypothesis suggested by Holdgate is that all these insects form a graded series with smaller and smaller "pinholes" in the wax layer; "but there are other possible systems which could equally well give a graded series of permeability and temperature dependence of diffusion" (26).

Wetting properties of the cuticle.—When contamination is excluded, the

structure of the cuticle has been shown by Holdgate (25) to be more important than chemical differences in determining the angle of contact between water and the cuticle surface; roughness tends to exaggerate the wettability of the cuticle if the angle is small, and, by trapping air, to cause an apparent increase in the angle if it is already high. Some aquatic insects, such as *Gyrinus marinus* Gyllenhal, maintain persistently large contact angles with water even though permanently submerged; they clearly do this by some active process, perhaps the continuous secretion of waxy material. If these insects are killed they behave like other insects when immersed in water: the contact angle is lowered. The nature of this change was studied by Holdgate (25) in the cockroach. If the labile components are removed by repeated washing, the cockroach cuticle behaves in the usual way when soaked in water: there is a marked fall in the angle of contact. But once this change has been brought about it is not reversed by desiccation of the cuticle. On the other hand it is reversed, and the contact angle rises again, if the cuticle is exposed in a moist atmosphere. Under these conditions the cuticle imbibes water and becomes flexible again. The rise in contact angle is probably attributable to a reorientation of polar molecules by the attraction of liquid water in the substance of the cuticle. These observations are important because an efficient waterproof layer of wax probably requires proper orientation. Beament (3) suggests that the presence of a water-saturated substrate, which will bring about the orientation of polar molecules, may be an essential part of this process.

There are striking changes in contact angles during the moulting cycle. The secretion of wax into the surface of the tracheae, or into the plastron of aquatic insects just before moulting, is probably important in the replacement of liquid by gas in these structures (69). This process will require the maintenance of a high retreating contact angle against water, as on the ordinary cuticle of *Gyrinus* (25). In *Rhodnius* the high contact angle immediately after moulting falls markedly when the cement layer is poured out, to rise again as the cement hardens. In the pupa of *Tenebrio* the contact angle increases greatly as the surface "bloom" of wax crystallizes on the surface. This change is chiefly a result of the roughness produced by the wax filaments.

DEPOSITION OF THE CUTICLE

Pore canals and cuticle formation.—During the enormous growth of the cuticle which takes place at engorgement in the tick, *Ixodes ricinus* (Linnaeus), the epidermal cells become greatly enlarged and rich in ribonucleic acid and alkaline phosphatase, changes associated no doubt with the production of the fibrillar proteins of the cuticle. The pore canals of the outer endocuticle remains patent, but those of the inner endocuticle are fully chitinized. Yet the new cuticular substance is deposited rapidly in the outer endocuticle. The materials for growth must therefore reach the outer layers through the substance of the inner endocuticle by a process of interssuscep-

tion (45). A similar growth mechanism is described by Picken (55) in the scales of Lepidoptera, and in the larval cuticle of *Sarcophaga* by Dennell (68).

More information about the function of the pore canals has been obtained by Way (67) in *Diataraxia*. The outer endocuticle in this larva is 7 to 10μ in thickness, the inner endocuticle 50μ or more. In the outer endocuticle the pore canals have a divergent tree-like form, but the inner endocuticle, the part laid down after moulting, is devoid of pore canals. A single pore canal runs to each of the tubercles of the epicuticle. This ending in the epicuticle is normally solid; but if the cuticle is treated with diaphanol, with 5 per cent potassium hydroxide or with pepsin, the aperture is disclosed. In the mature epicuticle the pore canal is thus blocked by a plug of fairly soluble material.

During the formation of the cuticle in *Diataraxia* the pore canals provide a channel for the transport of the moulting fluid. They also probably serve to transport the protein and dihydroxyphenol for hardening the cuticle. Thus, the longer the period during which the pore canals are in contact with the epidermis, the greater is the extent of the exocuticular hardening. Probably the hardening materials continue to be transported to the exocuticle for some time after ecdysis. On the other hand, recovery of waterproofing after abrasion occurs long after the pore canals are separated from the epidermis. They cannot therefore be responsible for secretion of waxes.

Ecdysial membrane and moulting fluid.—The moulting fluid has long been known to dissolve the old cuticle; in *Rhodnius* it contains a protease which will digest congo red fibrin inserted under cuticle (68), and it has been presumed to contain a chitinase. This chitinase in the silkworm (32) and in *Tenebrio* (31) has properties resembling that in the gut of *Helix*. In the pupa of the cecropia silkworm, as studied by Passonneau & Williams (53), the moulting fluid during the first two thirds of adult development is a dilute aqueous proteinaceous gel without action on the pupal cuticle. Then, at 14 days, it is converted to a sol, chitinase activity increases, and proteolytic activity appears. By 20 days the old endocuticle has disappeared.

During moulting in *Rhodnius*, if the old cuticle is removed, one can often see a thin moist pellicle beneath it, lying between the old skin and the new. This layer I have always regarded as having been delaminated from the old cuticle and as representing one or more laminae of the endocuticle which have become detached as a first step in digestion, the last of these laminae being commonly shed with the old cuticle before its digestion has been completed. A similar structure has been described by Passonneau & Williams (53) as appearing suddenly between the old cuticle and the epidermis at the outset of moulting. They call this the "ecdysial membrane" and state that it is eventually shed with the inner lining of the exuviae. This membrane has been further studied by Richards (60). It is only a small fraction of a micron thick and consists largely of chitin, like the endocuticle! He suggests that it is a discrete new cuticle secreted when the epiderma.

cells retract from the old cuticle, but its resistance to the moulting fluid is puzzling. It may be worth recalling that in *Rhodnius*, during the first few days of moulting, an inner layer is added to the existing cuticle before the epidermal cells become detached; this layer is subsequently digested (74).

Oenocytes and cuticle formation.—The only function suggested for the oenocytes, which has any evidence to support it, is that they are contributing lipoprotein or other components to the epidermis for incorporation into the epicuticle. The evidence for this view rests on the observation that in widely different insects (*Rhodnius*, *Tenebrio*) the conspicuous cycle of secretory activity in the oenocytes reaches its peak immediately before the lipoprotein of the cuticulin layer is deposited, and when first formed the staining reactions of this layer are the same as those of the contents of the oenocytes (68).

A similar cycle has been demonstrated by Wolfe (71) in yet another insect, the larva of *Calliphora erythrocephala*. Here again the peak in the secretory cycle of the oenocytes corresponds with the time of deposition of the epicuticle, and the secretory material shows similar staining properties to the epicuticle. Moreover, the oenocytes are connected with each other and with the epidermis by cytoplasmic bridges, through which secretory granules pass. In the crab, *Carcinus maenas* Pennant, there occur in the blood so-called "lipoprotein cells," derived from amoebocytes, which go through a similar cycle during the moult as the oenocytes of insects. They are believed by Sewell (64) to synthesize lipoprotein and transport it to the epidermis.

In the adult honey bee worker the oenocytes show their maximum activity at the same time as the wax-secreting epidermal cells are most active (39). That would be consistent with the idea that the oenocytes may be concerned in wax metabolism. Kramer & Wigglesworth also suggest (on somewhat inconclusive evidence) that the oenocytes may be concerned in the continuous production of wax by the integument of the cockroach *Periplaneta*. It is interesting to note that in *Calliphora* the oenocytes go through a secretory cycle during puparium formation similar to that occurring before moulting in the larva, and at this time wax is being set free on to the surface of the cuticle (73).

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THE COMPARATIVE MORPHOLOGY OF THE INSECT HEAD[1,2]

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The interpretations of the morphology of the head given by Snodgrass in his excellent review (25) and incorporated in his textbook (26) have been generally accepted by most students of the subject. They will therefore serve as the "baseline" for this review which will be limited chiefly to a critical analysis of some of the more important papers that have since added to our knowledge of basic morphology or have expressed views at variance with those generally accepted. Because of the limited space available no attempt will be made at a complete review of recent literature of the head or a complete analysis of the morphology of the head. It is regrettably necessary to omit many papers which do not advance new theories of basic morphology.

Recent contributions have been concerned chiefly with the segmentation of the head and the morphology of the facial region. These subjects will therefore be treated most fully. There have been two major reassessments of the morphology of the anterior region of the head. One, by Ferris and his associates, is based chiefly on the assumption that the clypeolabral, frontoclypeal, and "postfrontal" sutures mark the positions of intersegmental lines. The other, by DuPorte, regards these sutures as functional in origin and is based largely on the recognition of the importance of the hitherto neglected laterofacial inflections as an aid in interpreting the facial structures.

SEGMENTATION OF THE HEAD

Ferris (15) draws attention to the lack of general agreement on the segmentation of the head and adds that "not until this question is settled can real progress in the understanding of subsidiary problems be achieved." There is no disagreement now on the segmental constitution of the gnathocephalon but a wide divergence of opinion exists as to that of the protocephalon. The principal unsolved problems are (*a*) whether the region of the head derived from the embryonic cephalic lobes or blastocephalon is an unsegmented acron or is composed of two or more postoral metameres, and (*b*) whether or not there is an intercalary or second antennal metamere.

All modern concepts of head segmentation are based on the theory that arthropods are descended from an annelidan or protoannelidan stock. With the notable exception of Ferris and his associates, morphologists assume that this ancestral stock possessed an unsegmented preoral piece or prostomium followed by a series of postoral appendage-bearing metameres. It is univer-

sally agreed that some of the latter enter into the composition of the arthropod head, but there is no general agreement as to the number of metameres nor as to the contribution, if any, made by the prostomium.

Evidence from embryology.—Embryologists generally seem to be convinced that postoral metameres enter into the composition of the blastocephalon. Their evidence is based on one or more of the following observations: the presence of coelomic sacs, the association of permanent or transitory appendages with these sacs, the presence of neuromeres, and the mode of development of the brain. They depend most strongly on the presence of coelomic sacs. A pair of such sacs is normally associated with the antennae, and many authors have described a pair of intercalary or second antennal sacs between the antennal and mandibular sacs. Wiesmann (31) described two pairs of sacs anterior to the antennal in *Carausius morosus* Brauer. These, he assumed, indicate the presence of a labral and a preantennal segment. Preantennal sacs have not been described in any other insect but are known to occur in some Chilopoda and Crustacea. Labral sacs have been described by Mellanby (22) in *Rhodnius prolixus* Stål, by Roonwal (24) in *Locusta migratoria migratoroides* Reiche & Fairmaire, and by Miller (23) in *Pteronarcys proteus* Neumann. Roonwal claims further that in *Locusta* the brain develops from three pairs of lobes, two of which he believes to be neuromeres of a labral and a preantennal segment. Accepting the presence of coelomic sacs as evidence of postoral metameres in the head, these authors would claim that seven such metameres enter into the composition of the head: labral, preantennal, antennal, second antennal, mandibular, maxillary, and labial. This is the greatest number claimed by recent workers.

The presence of coelomic sacs is almost universally accepted as a criterion for identifying a metamere, but there does not seem to be any real foundation for the belief in a fundamental relation between coelomic sacs and metamerism. Consciously or unconsciously this belief may be based on the gonocoel theory of the coelom and of metamerism, according to which the coelom originated as a gonocoel and metamerism as a multiplication of the gonocoels. Actually nowhere among the Proterostomia does the mesoderm originate in the form of sacs. It originates as solid masses of cells, and in the annulates the first expression of metamerism is the segmentation of these solid strands. Their excavation to form sacs comes later and possibly represents a stage in the differentiation of the mesodermal derivatives, having no direct relation to metamerism. There seems to be no reason, therefore, why mesodermal excavations should not appear independently of metamerism. Indeed most coelomate groups are unsegmented.

It may be assumed, therefore, that the presence of coelomic sacs in the blastocephalon does not necessarily indicate that annelidan postoral metameres are incorporated into this region. Snodgrass (26) discusses this point in detail. Among other evidence he cites that advanced by various authors who have shown (*a*) that in Polychaeta prostomial sacs may develop as anterior extensions of the sacs of the first postoral metamere, though in *Scololepis fuliginosa* sacs are said to develop independently in association with

the palpi and (b) that a somewhat similar phenomenon occurs in *Limulus*, scorpions, and *Thelyphonus*. In scorpions the cephalic sacs are extensions of the cheliceral sacs and do not become completely shut off from them. In *Limulus* also there is at first a single pair of sacs extending from the cheliceral somite into the head lobe. Later an incomplete septum divides them into cheliceral and cephalic sacs, but the former soon disappear.

There is reasonably strong evidence therefore that coelomic sacs occurring in the blastocephalon may be secondarily developed, perhaps in association with transitory or permanent appendages. Therefore, the embryological evidence does not prove beyond all doubt that labral, preantennal, and antennal metameres, representing the first three postoral metameres of the annelid, enter into the constitution of the blastocephalon.

Evidence from comparative anatomy.—Ever since Claus in 1876 claimed that the first antennae in Crustacea are prostomial organs opinion has been sharply divided as to whether the regions bearing the eyes and antennae are prostomial or metameric in origin. Hanström (17), from his study of the extensive literature on the nervous system of annulates, came to the conclusion that the protocerebrum and deutocerebrum of insects are the equivalent of the dorsal portion of the brain in Polychaeta. This region of the brain develops in the trochophore by the concentration of nerve cells associated with the sense organs of the prostomium and is not part of the chain of metameric ganglia. It follows, therefore, that the eyes and antennae in insects are derived from prostomial organs and are not serially homologous with the metameric appendages. Assuming that the prostomium of Polychaeta is an unsegmented preoral region, the antennal region of insects and structures anterior to it cannot contain any postoral metameres.

Most recent writers regard the sutures of the head, with the possible exception of the postoccipital, as secondary and functional having no metameric significance. Ferris (13, 14), however, maintains that the principal transverse sutures are intersegmental lines marking the limits of labral, clypeal, occulo-antennal, mandibular, and maxillary segments. Evidence for this theory has been adduced in a series of papers by Ferris and Henry who have advanced a radical theory of head segmentation. They accept Hanström's claim of the prostomial origin of the eyes and antennae but maintain that the so-called prostomium of the Polychaeta is not a preoral structure but a dorsal lobe of the third metamere. The proboscis of these worms, hitherto regarded as stomodaeal in origin, is interpreted by them as consisting of three anterior body segments, the mouth being situated at the anterior end of the first segment and, therefore, apical in position.

The detailed evidence on which this theory is based is derived chiefly from Henry's (18, 19) comparative studies on the nervous system of the annulates. The nerves supplying the polychaete proboscis arise from the cerebral mass and the circumoesophageal nerve cord. Their origin and distribution are such that Henry concludes that they represent three pairs of segmental nerves. Comparing the cranial nerves of the arthropod with those of the polychaete proboscis and prostomium, she concludes that the three

segments of the proboscis are represented in insects by a labral, a clypeo-hypopharyngeal, and an occulo-antennal segment.

DuPorte (10) criticized this conclusion on the ground that, even if the theory of the three-segmented structure of the proboscis is accepted, some of her observations on the head of insects are susceptible of interpretations other than those given by her. The segmental nature of the proboscis has not been firmly established however. A basic assumption of Henry's theory is that the stomodaeal nerves always originate from the first segmental ganglion, and it is true that in the Oligochaeta, Onychophora, and Arthropoda these nerves arise either directly from the ganglion or from the stomodaeal commissure which, passing beneath the stomodaeum, connects the two halves of the ganglion. In these three groups of annulates there is a marked tendency for this commissure to unite in part with the circumoesophageal connectives. In the Polychaeta illustrated by Henry it is obvious that, except at their bases, these two nerve cords are completely and indistinguishably united. The nerves of the proboscis arise from the common trunk, and it is impossible to tell by topographical examination whether they originate in the connectives, in which case they may be segmental nerves, or in the commissures, in which case they are certainly stomodaeal nerves.

There is, therefore, as much evidence in Henry's work for the stomodaeal as for the metameric origin of the polychaete proboscis, and when we consider that all previous authors have agreed that it is an eversible region of the stomodaeum, we must conclude that the theory of Ferris and Henry is, at least, not proven.

Ferris and Henry deny the presence of a second antennal segment in any arthropod, maintaining that the second antennae, like the first, are prostomial organs. Henry's (19) description of the origin of the nerves in Crustacea certainly seems to confirm this view. The nerves of the second antennae arise in front of the ganglion from which the labral nerves, the stomodaeal commissure, and the stomodaeal nerves originate—the ganglion which can be identified with the tritocerebrum of insects. In the Branchiopoda they originate from the circumoesophageal connectives, but so do the nerves of the first antennae. In the other Crustacea illustrated by Henry they originate directly in the cerebral mass.

The tritocerebrum is generally interpreted as the neuromere of a second antennal segment, but Ferris and Henry maintain that it is the ganglion of the labral segment because it innervates the labral region. DuPorte (10) also questions the accepted interpretation, claiming that this is based on the position of the tritocerebrum between the antennal and the mandibular ganglia, and not on the actual tracing of its development in association with an intercalary segment. He thinks it more likely that it represents the prostomial ganglion as found in the Oligochaeta. In the Polychaeta nerve cell concentrations, associated with the sense organs, are added to it to form the archicerebrum of these animals. The brain of the insect probably contains no components other than those of the Polychaeta.

If the tritocerebrum is the ganglion of the second antennal segment, the

nerves of the crustacean second antennae should originate from it. If it is the ganglion either of the prostomium or of an apical labral segment these nerves should arise behind it and not in front of it.

Snodgrass (29, Fig. 49) illustrates the head of the isopod, *Lygida exotica* Roux, which is remarkably like that of an insect. The large second antennae arise meso-ventral to the eyes in exactly the same position occupied by the antennae in most generalized adult insects. The minute first antennae arise mesal to the second at the same horizontal level. Both pairs of antennae, in fact, are attached to the primitive median facial plate. If the second antennae are appendages of a postoral segment, they must in this species have migrated to the prostomial region.

The evidence for the prostomial origin of the second antennae seems, therefore, to be quite strong, but so many embryologists have described in the Arthropoda a second antennal segment clearly marked off from the blastocephalon by an intersegmental groove, and further defined by the presence of coelomic sacs, a neuromere, and appendage buds in series with those of the metameres, that it seems desirable at this time to withhold final judgment.

There is another possible explanation of the so-called second antennal segment which is suggested by the examination of some of the illustrations found in the literature of arthropod embryology. A very good example is Sollaud's drawings of the early stages of the decapod, *Leander serratus* Pennant, as reproduced by Snodgrass (27, Fig. 38). These show a well-defined "second antennal" segment in which the mouth opens and from which the labrum develops. The possibility suggests itself that this segment represents the first metamere or peristomium of the annelids through which the mouth opens. It would, therefore, be a "labral" segment, possibly having the tritocerebrum as its ganglion, but it would not be apical in position.

Sollaud's figures quite clearly show the second antennae as originating from this segment, but in the closely related *Palaemon*, as well as in other Malacostraca (19), the nerves of the second antennae most certainly do not arise from the tritocerebrum. This very tentative suggestion is not put forward as a theory but merely to suggest a problem that might repay investigation.

THE ECDYSIAL SUTURE

That portion of the ecdysial line which lies in the head is, typically, in the form of an inverted Y. The stem or coronal suture runs along the midline of the vertex, and the arms run latero-ventrally[3] down the face, forming the V or U-shaped frontal suture. These can be distinguished from other facial sutures by the fact that they are cleavage lines along which the pigmented exocuticle does not develop and, as a result, they appear as clear pale lines in the pigmented head. They can be further distinguished by the fact that they are not in the form of grooves or sulci associated with internal ridges (9, 28).

[3] Terms denoting position or direction refer to the primitive hypognathous orientation.

They are developed in the heads of most immature insects but are lacking in most adult heads though they persist in some of the lower forms.

This Y-shaped suture has long been known as the epicranial suture, but several different sutures have been confused under this name. A midcranial inflection often carries the coronal suture inwards, and the sulcus thus formed has been mistaken for the coronal suture. This sulcus may continue ventrally beyond the coronal suture and in extreme cases may meet the frontoclypeal sulcus. In some Coleoptera this combination of midcranial and frontoclypeal sutures has been called the epicranial suture, and in the heads of many adult insects vaguely defined grooves have been interpreted as parts of the epicranial suture. Because of this confusion it is suggested (9, 28) that the use of the term "epicranial suture" be discontinued and that "coronal" and "frontal" suture should be used only for the stem and arms of the ecdysial line or suture.

Snodgrass at first followed Crampton in distinguishing between frontal and postfrontal sutures, the first passing mesal, the second lateral to the antennae. Ferris (13) claims that a frontal suture, as thus defined does not exist but retains the term "postfrontal" suture for that which passes between the compound eyes and the antennae. When the cleavage lines pass mesal to the antennae he interprets them as frontoclypeal sutures. Cook (5 to 8) follows him in this and has confused the frontal and frontoclypeal sutures in many larval heads.

DuPorte (9) and Snodgrass (28) showed that the frontal and postfrontal sutures are not two distinct structures but the two arms of the ecdysial suture following different courses. Snodgrass lists five different paths that they might follow but claims that in every case they pass between that portion of the cranial wall from which the mandibular and maxillary muscles originate and that from which the facial muscles originate. As will be shown later there are exceptions to this generalization.

Ferris (13, 14) rejects the interpretation of the "postfrontal" suture as merely a functional cleavage line. He believes that it formed primitively the intersegmental line between an oculo-antennal segment and the mandibular segment, but when the ocular lobes increased in size and met in the midline it became the suture between the antennal region and the ocular lobes. Later (16) he states that this suture can be traced back to the head lobe of the Polychaeta where

it seems to indicate a separation between the ocular and antennal portions of what in insects and other arthropods may be called the antennal-ocular segment of the head capsule. . . . The first thought that occurs is that the head lobe is actually composed of parts of two segments but nothing can be seen yet which definitely supports this idea except the persistence of this suture.

There can be no doubt that Ferris' postfrontal sutures are the lateral cleavage lines of the face. These lines frequently pass mesal to the antennae, which are therefore borne on the same sclerite as the eye. If his "first thought" is correct it must be assumed that in such insects there has been an "adaptational shifting" of the antenna from its own segment to that of

the eye—an assumption which he would be the first to denounce as being "on the intellectual level of a belief in witchcraft."

If the frontal suture has the morphological significance ascribed to it by Ferris, and is not simply a functional cleavage line, it is difficult to explain why it is almost invariably present in immature insects and, with relatively few exceptions, absent in adults. Besides, if it is an intersegmental line which can be traced back to the Polychaeta, it would be expected to persist in arthropods other than insects. His identification of this suture with a sulcus in the head of *Scutigerella* is, as Snodgrass (28) has shown, extremely doubtful.

INFLECTION OF THE FACIAL REGION

The sutures of the face, other than the ecdysial, are grooves or sulci marking the positions of internal ridges or inflections. These ridges, as well as the anterior tentorial arms, all converge on the anterior mandibular articulations, and DuPorte (9) has suggested that they are a means of reinforcing the face against the pull of the mandibles. Indeed most modern authors (2, 9, 26, 30) regard them as secondary reinforcements along lines of stress in the cranial wall. Bigelow (2) emphasizes the importance of the tentorium which braces the head and provides a rigid base from which motion can be transmitted to the mouthparts, foregut, and antennae. He believes that stresses applied to the tentorial arms by the muscles attached to them are transmitted to the facial integument between the tentorial pits and that this accounts for the origin of the frontoclypeal inflection which reinforces this region.

The laterofacial inflections.—Probably most generalized insects have a pair of sulci extending dorsally from the anterior mandibular articulation to a point between the antennae and compound eyes. These sulci have been variously designated subantennal, subocular, or frontogenal sutures. They mark the position of internal frontogenal inflections or ridges (Fig. 1, *Fgi*).[4] Ferris (13) suggested that these sutures were formed between the primitive median facial plate of apterygote insects and the lateral regions of the head when the latter grew ventrally to form the genal regions of the pterygote head. DuPorte (9) agreed with this interpretation and showed further that in *Lepisma* an inflection, which will later contribute to the frontogenal ridge, is already present as an infolding of that portion of the lateral edge of the facial plate to which the mandible is attached by membrane. This fold extends to the secondary articulation which the mandible makes with the face, and when the lateral regions of the cranium descend ventrally, bringing the primary articulation to the same horizontal level as the secondary, the lateral facial fold forms the inner lamella of the frontogenal inflection.

In *Lepisma* the anterior tentorial pit lies in the frontogenal fold immediately dorsal to the anterior mandibular articulation, and in most pterygote insects it seems to retain this position in the frontogenal sulcus. In a

[4] For explanation of abbreviations see list given on page 70.
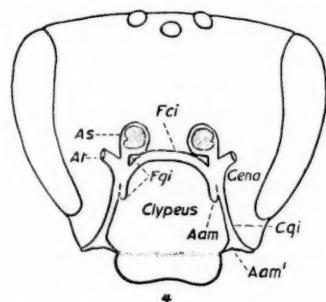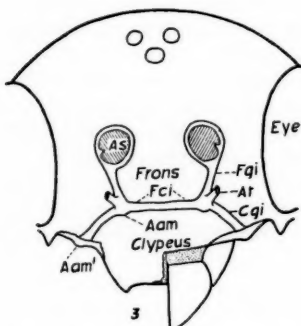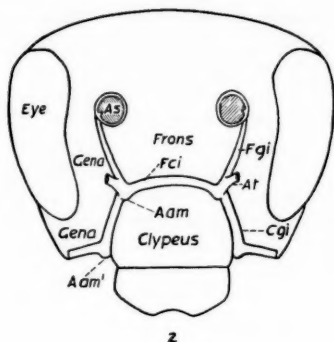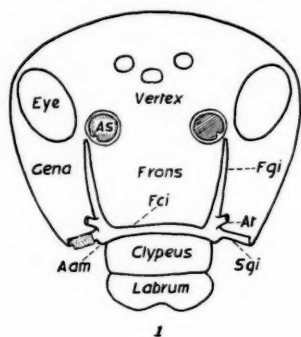
FIG. 1. Facial inflections in the generalized Pterygota (schematic).

FIG. 2. Facial inflections in the generalized Hymenoptera (schematic).

FIG. 3. Facial inflections in *Ametastegia equiseti* (Tenthredinidae).

FIG. 4. Facial inflections in *Apis mellifera* (Apidae).

few insects it migrates to the frontoclypeal suture, but Snodgrass' contention that it always lies in this suture is probably attributable to the confusion between the frontoclypeal suture and the epistomal suture in higher insects, which will be discussed later.

The ventral end of the frontogenal inflection forms the condyle of the anterior mandibular articulation (Fig. 1, *Aam*). Dorsally the inflection terminates, typically, near the lateral edge of the antennal socket (Fig. 1, *As*) but, because of the variability in the position of the antennae, this relation is not always retained.

The genal regions, in many of the higher insects, have continued their ventral growth beyond the level of the mouth, and their mesal edges have united with the previously free lateral edges of the clypeus (2, 12). When this happens a pair of clypeogenal inflections (Fig. 2, *Cgi*) develops con-

tinuous with the frontogenal inflections, and the laterofacial inflections now consist of two components, the frontogenal and clypeogenal. This fact was not recognized by DuPorte in his earlier work (9) and, consequently, some of the inflections which he there interprets as frontogenal are either the clypeo-genal or the composite laterofacial. These inflections are frequently reduced and sometimes completely absent, but are nevertheless among the most constant inflections of the facial region.

Ferris (14) claims that the anterior tentorial pit lies in a "tentorial su-ture" which cuts off a small sclerite, the paraclypeal lobe, lying lateral to the clypeus. The claim is based on the structure of the face in larval Cory-dalidae where the frontal suture traverses the clypeal region and divides it into a median and two lateral lobes. Ferris interprets the frontal suture as the frontoclypeal and, therefore, believes that the lateral lobes lie outside the clypeal region. His "tentorial sutures," in the mesal ends of which the tentorial pits open, lie dorsal to the lateral lobes. These sutures are inter-preted by other authors as lateral portions of the frontoclypeal suture, but DuPorte (9) believed them to be frontogenal sutures which, because of the growth of the frontal region, have been forced into the horizontal position normally occupied by the frontoclypeal suture. Either of these interpreta-tions may be correct or the sulci may be clypeogenal sutures. In any case the lateral lobes are parts of the clypeal region. Cook (6, 7, 8) has identified Ferris' tentorial suture or paraclypeal fold in the heads of a number of in-sects, but, at least in some cases, the suture thus identified is the same as DuPorte's laterofacial suture and the paraclypeal lobes are part of the frons as defined below.

The frontoclypeal inflection.—The frontoclypeal inflection (Fig. 1, *Fci*) is a transverse ridge in the median facial region. Its extremities unite with the frontogenal inflections between the anterior tentorial arms (*At*) and the anterior mandibular condyle (*Aam*). It probably developed as a strengthen-ing ridge to reinforce the cranial wall when the secondary mandibular articu-lation was established. It lies at the level of the primary mouth and thus divides the median facial region into a postoral[5] frons and a preoral clypeus (9, 12).

It is interesting to note that in some Crustacea such as the isopod *Lygida* (29, Fig. 49), in which the lateral regions of the head have grown ventrally and the mandible has made a secondary articulation with the face, inflec-tions identical with the frontogenal and frontocylpeal inflections of insects have developed. The former terminate at the lateral edges of the second antennae.

The subgenal inflection.—The genal regions, in the generalized pterygote, have descended to the level of the mouth, bringing the primary mandibular articulations to the same level as the secondary ones. Between the two articu-lations the base of the mandible is attached by membrane to the ventral

[5] "Postoral" and "preoral" are used here to denote respectively the area dorsal and that ventral to the mouth in the hypognathous head, without reference to the morphological position of these areas.

edge of the gena which is often folded under to provide additional strength to the cranial wall (Fig. 1, *Sgi*). Sometimes a narrow sclerite, the pleurostoma, develops between the edge of the gena and the base of the mandible. When this happens a subgenal sulcus is formed between the gena and pleurostoma marking the position of the subgenal inflection (9).

The Frontoclypeal Region

The clypeus.—The identification of the clypeus or clypeal region presents no difficulty in most generalized insects, because it projects beyond the mouth as a free lobe in the sense that it is not united laterally with any other cranial sclerite. In most of the higher insects it is united laterally, for all or part of its length, with the mesal edges of the genae and is bounded laterally and dorsally by a U-shaped epistomal suture. This suture is usually interpreted as the frontoclypeal suture which has arched upwards into the frontal region carrying the anterior tentorial pits with it. Snodgrass claims, however, that the portion of the alimentary tube lying in the clypeal region is the preoral cibarium, which is tantamount to saying that the primary mouth has accompanied the suture in its dorsal migration.

Because of the position of the tentorial pits in the epistomal suture, DuPorte (9) claimed that the vertical components of the suture are frontogenal sutures and the horizontal component a transfrontal suture, therefore the sclerite is an antefrons and not a clypeus. Snodgrass later (28) presented convincing evidence that the sclerite is the clypeus, and DuPorte & Bigelow (12) showed that, in the Hymenoptera, there is no dorsal movement of the frontoclypeal suture, the mouth, or the tentorial pits. There is instead, according to these authors, a further ventral extension of the genae on either side of the clypeus, and the mesal edges of the genal extensions unite with the lateral edges of the clypeus. At the lines of union a pair of clypeogenal inflections (Fig. 2, *Cgi*) develop, and these are marked externally by corresponding sulci which form the vertical components of the epistomal suture. The horizontal component of the suture is the frontoclypeal sulcus in its primitive position. The ventral extremities of the frontogenal sutures, containing the tentorial pits, also contribute to the epistomal suture.

The term "epistomal suture" is commonly used as a synonym of "frontoclypeal suture." To avoid coining a new term, it is suggested that this term be retained for the suture bounding the clypeus, with the understanding that in many insects the frontoclypeal suture forms only a part of it.

With the descent of the genal regions and the formation of the clypeogenal inflections the anterior mandibular articulations (Fig. 2, *Aam'*) shift to the ventral ends of the inflections, which now form part of the system of reinforcing ridges.

These developments in the head of the Hymenoptera can be easily followed by comparing Figure 2 with Figure 1. The frontogenal inflections are often obsolescent or completely lost. When present they seem to terminate always in the antennal ridges. Their length, therefore, depends on the extent to which the area between the antennal sockets and the frontoclypeal suture,

that is to say the frons, is reduced. In *Ametastegia equiseti* (Fallen) the frons is somewhat reduced, but the inflections (Fig. 3, *Fgi*) are well-developed. In *Apis mellifera* Linnaeus, in which the antennal sockets lie against the frontoclypeal suture, the frontogenal inflections (Fig. 4, *Fgi*) are reduced to mere stumps. It will be noted also that in the more primitive *Ametastegia* the genae have descended only part way down the clypeus, while in *Apis* they have descended the full length of the clypeus.

The frons.—The frons is usually defined as the area dorsal to the clypeus and bounded by the frontoclypeal and frontal stutures. As thus defined, it is very variable, and, except in larval insects, usually indeterminate. Snodgrass tried to fix its limits more definitely by defining the frontal (as opposed to the postfrontal) suture as the arms of the epicranial suture which "diverge above the median ocellus and proceed ventrally on the face, *mesad* of the antennal bases, toward the anterior articulation of the mandible." Later (28), however, he abandoned this interpretation, agreeing with DuPorte (9) that there is no constancy in the position of the frontal suture. Nevertheless he would retain the term "frons" for an indefinite region, the general area dorsal to the clypeus or clypeal region. If this definition is accepted, four familiar terms used for cranial regions (frons, vertex, genae, and parietals) would all be indeterminate and of no morphological significance.

DuPorte's (9) concept of the frontoparietal region (ignoring the cleavage lines) is that of a single sclerotized plate whose ventral region is partially divided by the frontogenal inflections into a median and two lateral lobes (Fig. 1). He defines the median lobe as the frons, the lateral lobes as the genae and the undivided dorsal region as the vertex. Thus these terms have the same morphological significance in all insects. He suggested that when the frontal suture is present, that portion of it lying dorsal to the area between the frontogenal sutures should be regarded arbitrarily as the dorsal limit of the frons, but because of the great variation in the course of the frontal suture this suggestion is not acceptable. In some insects, however, a transverse suture develops between the dorsal extremities of the frontogenal sutures, forming a dorsal boundary to the frons.

It might be argued that, because of the established use of the term "frons" in the taxonomic literature in the sense accepted by Snodgrass, new terms should be coined for the three lobes of the frontoparietal region. In this review, however, the term "frons" is used for the median postoral lobe lying dorsal to the clypeus and bounded laterally by the frontogenal sutures. The terms "frontal apotome" and "frontoclypeal apotome" have been used by Snodgrass for the area cut off at ecdysis by the lateral cleavage lines. If it is necessary for descriptive purposes to use a general name for the region enclosed by the frontal suture in the intact head it may be called the facial apotome.

Criteria for identifying the frons and clypeus.—When the facial sutures are not all obviously present in their typical positions, it may be difficult to identify the facial regions. Snodgrass (28) claims that the cibarial dilators always originate in the clypeus, while the anterior pharyngeal dilators which

are inserted within the loops formed by the frontal ganglion connectives, originate in the frons. He claims further that the frontal ganglion always retains its position at the level of the primary mouth. The position of the mouth being thus fixed the approximate limits of the frontal and clypeal regions can be determined. DuPorte & Bigelow (12) showed that, in the Hymenoptera at least, the tentorial pits retain their position at the level of the mouth, regardless of changes that take place in the structure of the head.

The second of these criteria may be the most constant. Muscles do change their origins and the tentorial pits may migrate dorsally or ventrally. Nevertheless all of these criteria are often of practical value.

The frontoclypeus in the Lepidoptera.—Space does not permit a full discussion of the interpretation of the frontoclypeus by recent authors, but a brief consideration of the facial structure in the Lepidoptera and in larval Culicidae will illustrate the confusion that arises when there is no clear morphological concept of this region.

The inverted Y-shaped sulcus in the head of larval Lepidoptera was for a long time interpreted as the epicranial suture, and the triangular sclerite embraced by it as the frons. Snodgrass claimed that the V-shaped arms of the sulcus form the frontoclypeal suture, and the sclerite is consequently the clypeus. DuPorte (9), basing his interpretation on the association of the tentorial pits with the suture, believed the arms to be frontogenal sutures and the sclerite an antefrons. Hinton (20), applying the criterion of muscle attachments, interprets the sclerite as a median region only of the frontoclypeus. He believes that the sulcus, which he calls the adfrontal suture, is peculiar to caterpillars and cuts through, but does not bound, the frontoclypeus.

Applying the three criteria mentioned above, DuPorte (11) showed that the frontal ganglion (Fig. 5, *Fg*) lies approximately at the same level as the tentorial pits (*At*), that the cibarial dilators (Fig. 5, *1, 2, 3*) originate in the sclerite ventral to the tentorial pits, and that two of the three pairs of pharyngeal dilators (Fig. 5, *4, 5*) originate in the sclerite dorsal to the pits. He concludes that the sclerite is the complete frontoclypeus, that the area ventral to the pits is the clypeal region, and that dorsal to the pit the frontal region. The arms of the V-shaped sulcus he now believes to be laterofacial sutures the dorsal ends of which have been drawn together by the very deep midcranial inflection of the vertex (Fig. 5, *Mci*).

There is a large quadrangular sclerite in the head of most adult Lepidoptera, bounded ventrally by the clypeolabral suture, laterally by a pair of vertical sutures in which the tentorial pits open, and dorsally by a transverse suture between the antennal sockets. It is usually referred to as the clypeus, sometimes as the frontoclypeus. The position of the tentorial pits and the origin of the facial muscles vary in different species, but a comparison of the internal view of the face in *Danaus archippus* Fabricius (Fig. 6) with that of a generalized hymenopteron (Fig. 2) leaves no doubt that the vertical ridges (*Fgi+Cgi*) are identical in the two and that the sclerite in
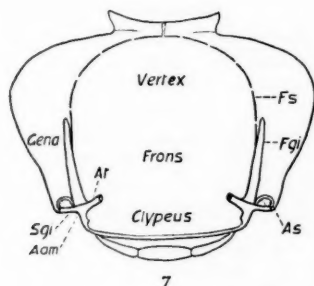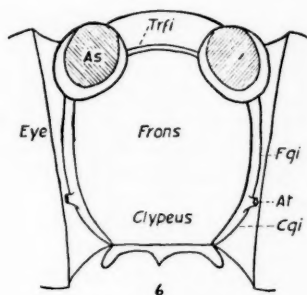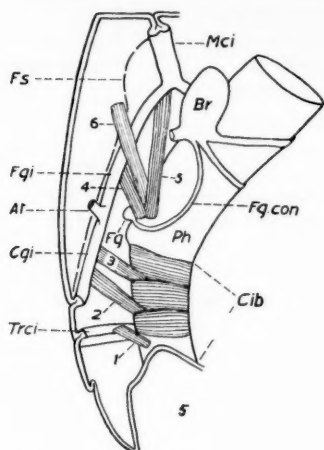
FIG. 5. Head of larva of *Protoparce quinquemaculatus*, showing facial inflections and muscle origins.

FIG. 6. Facial inflections of *Danaus archippus*, adult.

FIG. 7. Facial inflections of *Aedes* sp. (Culicidae), larva.

Danaus is a frontoclypeus, the homologue of the triangular sclerite plus the membranous anteclypeus in the larva.

The frontoclypeus in culicid larvae.—The heads of larval mosquitoes have a short coronal suture from which the branches of the frontal suture (Fig. 7, *Fs*) diverge widely to enclose a large sclerite, the facial apotome, which occupies most of the anterior region of the head and extends ventrally to the labrum. A similar frontal suture is found in the heads of many other nematocerous larvae, and Anthon (1) regards the entire apotome as the frons which has grown ventrally and forced out the clypeus. Cook (7), on the other hand,

interprets the cleavage lines as the frontoclypeal suture and the apotome as the clypeus. Snodgrass (28) interprets the apotome as the frontoclypeus.

In the culicid larva a pair of sulci, lateral and parallel to the cleavage lines, extends from the anterior mandibular articulations (Fig. 7, *Aam*) dorsally to a point about half-way up the face. They mark the position of an internal ridge (*Fgi*), to the ventral ends of which the tentorial arms (*At*) are attached. Cook calls these sulci paraclypeal folds, identifying them with Ferris' tentorial sutures. DuPorte (9), on the other hand, regards them as frontogenal sutures, and a comparison of Figures 1 and 7 shows clearly that the inflections associated with the sutures in the two figures are identical. Obviously, therefore, the median lobe projecting free beyond the mandibular articulations is the clypeal region, the area between the frontogenal sutures is the frontal region, and the area of the facial apotome dorsal to this is a median area of the vertex.

Origin of the facial muscles.—Snodgrass (28) claims that the cibarial dilators always originate in the clypeus, that the anterior dilators of the pharynx originate in the frons, and that these and other facial muscles originate between the cleavage lines. Numerous observations prove that this rule is generally true, but muscles are purely functional structures which commonly change their attachments in the interest of mechanical efficiency. It is not surprising, therefore, that there are many exceptions to Snodgrass' generalization.

Differences in muscle origins in relation to the cleavage lines may appear in species of the same family. In the larva of the tipulid, *Holorusia rubiginosa* Loew, all of the facial muscles originate between the cleavage lines (8). Chiswell (4) finds this to be true of the larvae of *Dictenidia bimaculata* Linnaeus but not of *Tipula paludosa* Meigen or *Tipula irrorata* Macquart, in both of which the cibarial dilators, the pharyngeal dilators, and the oral arm retractors originate lateral to the cleavage lines.

In the larvae of Trichoptera (20) and Lepidoptera (11, 20) a pair of pharyngeal dilators (Fig. 5, 6) originates lateral to the cleavage lines. The muscle origins in the adult head of *Danaus archippus* conform to Snodgrass' rule, but in *Protoparce quinquemaculatus* Haworth a pair of cibarial muscles originate in the frontal region in exactly the same location as do the pharyngeal dilators in *Danaus*, while another pair of cibarial dilators and the pharyngeal dilators originate at the same level in the vertex (11).

THE OCCIPITAL REGION

The primary articulations of the mouth appendages are made with the occipital region which must, therefore, be derived largely, if not entirely, from the gnathal segments. Typically this region is separated from the parietal by the occipital suture which extends up the sides and frequently across the top of the head, terminating on each side just in front of the primary mandibular articulations. The suture may be a secondary strengthening ridge in association with the mandibular articulation, but Ferris (13, 14), who calls it the premandibular suture, interprets it as the intersegmental

line between the oculo-antennal and the mandibular segments. Its position and a comparison with certain primitive arthropodan heads suggest that this may be a valid interpretation, but with few exceptions, embryological studies do not confirm the persistence of this intersegmental line in the developing head. More critical study of the development of the head may solve this question.

The posterior tentorial pits lie in the ventral extremities of the postoccipital suture which divides the occipital region into an anterior occipital arch and a posterior postocciput. The pits develop, in the embryo, between the maxillae and the labium; therefore the suture is generally regarded as the persistent intersegmental line between the maxillary and labial segments. The postocciput on this interpretation is a dorsal region of the labial segment, and the occipital arch is derived from dorsal regions of the mandibular and maxillary segments.

Ferris (13) disagrees with this interpretation. He suggests instead that the suture is the intersegmental line between the mandibular and maxillary segments, that the postocciput belongs therefore to the maxillary segment, and that the labial segment contributes nothing but the submentum and gula to the sclerotized cranium.

Kelsey (21) has followed Comstock & Kochi in suggesting that the occipital and postoccipital sutures are pleural sutures of the mandibular and maxillary segments. Since the postoccipital suture always, and the occipital commonly, extend around the cranium, this theory would seem to derive all or most of the occipital region and an undefined area in front of it from subcoxal elements of the mandibular and maxillary appendages, but there is no authenticated embryonic evidence that such subcoxal elements play any part in the formation of the cranium.

THE SUBMENTUM

Formerly regarded as the united bases of the second maxillae, this sclerite has been interpreted by several authors as derived from the ventral region of the labial segment. Snodgrass suggested that it may contain elements of both the labial sternum and the cardines of the appendages. Ferris (13) believes that it is derived solely from the body wall of the labial segment.

Roonwal (24) claims, on the other hand, that in the embryo of *Locusta* the submentum is derived entirely from the appendages and that the labial sternum plays no part in its formation but unites instead with the mandibular and maxillary sterna to form the hypopharynx. Chaudonneret (3) also, largely on the evidence of the musculature, concludes that the submentum in Thysanura is formed from the united subcoxae of the second maxillae.

The fact that the submentum is adnate to the cranium suggests that it is part of the body wall, but there are well-established precedents for the incorporation of subcoxal elements in the body wall. This is one problem which can be solved by embryological evidence; therefore if Roonwal's observations are correct there can be no doubt of the appendicular origin of the submentum, but confirmation, in other insects, of these observations is desirable.

ABBREVIATIONS USED IN THE FIGURES

All figures are redrawn, with slight modifications, from DuPorte (9, 11) and DuPorte & Bigelow (12).

Aam	Anterior mandibular articulation in the generalized pterygote	*Fg*	Frontal ganglion
		Fg.con.	Frontal ganglion connective
		Fgi	Frontogenal inflection
Aam'	Anterior mandibular articulation in Hymenoptera	*Fs*	Frontal suture (cleavage lines)
		Mci	Midcranial inflection
As	Antennal socket	*Ph*	Pharynx
At	Anterior tentorial arm	*Sgi*	Subgenal inflection
Br	Brain	*Trci*	Transclypeal inflection
Cgi	Clypeogenal inflection	*Trfi*	Transfrontal inflection
Cib	Cibarium	*1, 2, 3*	Cibarial dilators
Fci	Frontoclypeal inflection	*4, 5, 6*	Anterior pharyngeal dilators

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CYTOGENETICS AND SYSTEMATIC ENTOMOLOGY[1]

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Chromosome cytology has contributed to insect systematics in several different ways which are not always distinguished very clearly by non-cytologists. Firstly, it has been shown that several quite distinct types of genetic systems characterise certain major groups (orders, families, etc.) of the Insecta. For example, male Hymenoptera are genetically haploid, while those of the higher Diptera are diploid, but generally lack crossing-over (whereas in most other groups of insects crossing-over occurs in both sexes). The cytological mechanisms of sex determination vary greatly within the Insecta. In certain groups such as the aphids special cytogenetic mechanisms control the complex alternation of sexual and parthenogenetic generations. And the gall midges (Cecidomyidae) have two distinct kinds of chromosomes, both of which are present in the nuclei of the germ line, but one kind of which are eliminated from the somatic nuclei early in embryology.

Such differences in genetic mechanisms are obviously of great significance in population genetics and evolution and in many instances also in physiological genetics. They are not merely morphological characters like those (mouth-parts, wing venation, etc.) commonly used in defining systematic categories, since they concern the whole of the genetic apparatus on which speciation, evolution, and adaptive radiation depend.

The amount of genetic recombination varies greatly from species to species and group to group, depending on the number of chromosomes and the average number of portions into which they are broken at each generation by meiotic crossing-over. Some scale insects and Mallophaga with only two pairs of chromosomes and few chiasmata (points of crossing-over) must exhibit very "tight" linkage, while certain Lepidoptera with over a hundred chromosome pairs probably have fifty to a hundred times as much genetic recombination.

If we knew just how the major differences in genetic mechanisms had arisen in the course of evolution, we might use this knowledge in establishing the relationships of the insect orders and other "higher categories" on a firmer foundation. But in most cases the origins of the main types of genetic systems are as unknown as the origins of the groups themselves.

Secondly, cytologists are concerned with the cytotaxonomic differences which exist between related species. These only rarely involve major differences in the genetic mechanism, but often consist of differences in chromosome number or in the sizes and shapes of some of the chromosomes in the

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[1] The survey of the literature pertaining to this review was completed in May, 1956.

set. Such differences may sometimes be used to distinguish "sibling" or cryptic species that cannot be separated at all, or only with difficulty or uncertainty, on external characters. They are the result of chromosomal rearrangements which have arisen spontaneously and established themselves in phylogeny. One type of rearrangement (called a centric fusion or simply a fusion) leads to a diminution in chromosome number, while another ("dissociation") produces an increase. "Deletions" lead to loss of genes, "duplications" to acquisition of extra genetic material, "inversions" to changes of gene-sequence within the individual chromosome, and "translocations" to interchanges of genetic material between nonhomologous chromosomes.

All these types of rearrangments depend on a few simple laws or principles which no doubt arise from the actual molecular structure of the chromosome itself, so that they probably do not vary appreciably from group to group. Credit for establishing these principles must go largely to Muller (47 to 50).

The first principle is that chromosomes do, from time to time, undergo spontaneous breakage. In order to be of any significance in future generations such breaks have to occur in the germ-line of an individual which happens to leave some descendents. Freshly-broken chromosome ends seem to be indiscriminately "sticky," i.e., any freshly broken end is capable of joining with any other such end. Natural chromosome ends ("telomeres") are, however, not "sticky," i.e., they will not fuse, either with one another or with freshly-broken ends. Thus a telomere cannot assume an interstitial position in a chromosome, and neither can a freshly-broken end become converted into a telomere. These principles, first developed as a result of work on induced rearrangements in laboratory stocks of *Drosophila*, are supported by all critical studies of natural evolutionary changes of chromosome structure.

In most groups each chromosome has a single short region ("centromere" or "kinetochore") which serves to attach it to the spindle at mitosis. Chromosomes lacking centromeres may be produced in genetical experiments but they cannot persist through a series of cell-divisions. And, similarly, chromosomes with two or more centromeres are liable to become disrupted through two centromeres in the same strand passing to opposite poles at anaphase. Thus in most groups only "monocentric" chromosomes can survive. Ring-chromosomes, which have a centromere but no telomeres, can be obtained in experiments and maintained in some stocks of *Drosophila* but do not exist in natural species, presumably because they become disrupted at mitosis or meiosis.

In some groups of insects, however, the chromosomes do not seem to have localized centromeres. According to one view such chromosomes have a "diffuse centromere activity" which attaches them to the spindle along their entire length; according to another they are "polycentric," i.e., have many centromeres distributed along the chromosome at intervals.

The consequence of these facts for systematic studies is that differences in chromosome number between species are not attributable to reduplica-

tion or loss of whole chromosomes having occurred (except perhaps in a few special cases). Neither are they a result of simple chromosomal fragmentation (one chromosome breaking into two) or simple fusion (which would involve the joining together of telomeres). Only rearrangements involving at least two breaks and a rejoin (usually two rejoins) can give rise to new chromosomes.

Even if the new chromosomes produced by rearrangement are capable of persisting through a number of cell-generations and do not lead to loss of essential genes they are still liable to be eliminated from the population as a result of natural selection unless individuals possessing them in the heterozygous condition have an adaptive superiority over those lacking them. Thus every cytotaxonomic difference between species is necessarily preceded by a condition of cytological polymorphism in which the "old" and the "new" chromosomes occur alongside one another in the population. And, in fact, there are many cytologically polymorphic species of insects in which the alternative types of chromosomes co-exist with one another in a state of flux, exhibiting seasonal or microgeographic changes in their relative frequencies. Often the heterozygotes exhibit heterosis (having a higher adaptive value than either of the homozygous types) so that we get a balanced rather than a transient polymorphism. Except for occasional aberrant individuals in normally monomorphic species, naturally occurring chromosomal polymorphisms, whatever kind of rearrangements they are attributable to, are probably in all instances adaptive and in fact furnish the basis for plastic adaptive mechanisms of great importance in population genetics. This is because, in practice, we can only expect to find in nature the polymorphisms which are sufficiently well balanced to have persisted under natural selection. Not all such polymorphisms will lead eventually to cytotaxonomic differences between species, but all cytotaxonomic differences must have arisen from chromosomal polymorphisms. Thus the principles of population genetics provide a basis for appreciating the meaning of cytotaxonomic differences. And, conversely, the study of chromosomal differences between species is not merely a matter of counting chromosome numbers or evaluating chromosome shapes as taxonomic characters but leads directly to consideration of problems of population genetics at the present time and in the past.

In a few species of parthenogenetic insects which lack males, evolution of chromosome sets by polyploidy has taken place (see p. 77), but there is no evidence that this process has played any part in the evolution of sexually-reproducing insects or in forms which have an alternation of sexual and parthenogenetic generations. However, genetically diploid or haploid insects always seem to have many of their somatic cells polyploid, because of multiplication of chromosome sets in the course of development and differentiation.

The extent to which cytotaxonomic differences between species can in practice be detected varies with the material. In those Diptera which have

giant salivary gland ("polytene") chromosomes (e.g., Sciaridae, some Ceci-
domyidae, Chironomidae, Simuliidae, Drosophilidae, some Agromyzidae,
and no doubt members of other families not yet examined) all cytotaxo-
nomic differences, however minute, should, in theory, be observable. In other
orders of insects, however, and in those Diptera whose salivary chromosomes
are unsuited for detailed analysis, only certain types of differences in gene-
sequence are detectable cytologically, for example those which lead to
changes in chromosome number or shape, and most of the minute differences
escape observation. Thus the cytotaxonomic differences which we observe
between the chromosomes of species of beetles or grasshoppers are only a
fraction, and perhaps a small fraction, of those which exist.

We can make a useful distinction between two types of monocentric
chromosomes. Those which possess two limbs of equal or subequal length,
separated by the centromere (V-shaped or J-shaped bodies at metaphase)
are called metacentric. Those which have one limb extremely minute and
much shorter than the other are called acrocentric. Frequently the second
arm of an acrocentric chromosome is difficult or perhaps impossible to detect
under the microscope, but it is probably always present, i.e., naturally
occurring chromosomes never have the centromere in a strictly terminal
position. The distinction between metacentric and acrocentric chromosomes
is not an absolute one, because intermediates occur in some species which are
difficult to assign to either category. But the distinction is of practical
importance when we come to consider the various types of rearrangement
which have occurred in evolution. Some species have all their chromosomes
of one type, while others have a chromosome set ("karyotype") composed
of some acrocentric and some metacentric chromosomes.

Two main types of chromosome segments which appear quite different
in thickness and degree of condensation at various stages of the division
cycle are termed euchromatic and heterochromatic. Some heterochromatic
regions have only rather slight genetic activity and are said to be genetically
inert or subinert (the inertness is probably only relative). There are, how-
ever, several different kinds of heterochromatin (i.e., chromosomal material
showing this differential condensation), and it may be that there is a con-
tinuous range of "chromatins" connecting the extremes usually recognized
as euchromatin and heterochromatin. Both X and Y chromosomes are
frequently composed wholly or in part of heterochromatin, and in insects at
any rate all chromosomes probably contain some heterochromatic regions,
usually adjacent to the centromere and the telomeres.

THE MAJOR TYPES OF GENETIC SYSTEMS IN INSECTS

We know very little about the chromosome cytology of the Apterygote
orders of insects. The earliest winged insects were probably forms with a
moderate number of relatively large monocentric chromosomes (as in pres-
ent-day Ephemeroptera, Odonata, Plecoptera, Embioptera, and the Orthop-
teroid groups). They almost certainly had male heterogamety (as in all the

groups mentioned above), and meiosis was most likely quite "normal" in both sexes, with chiasmata in oogenesis and spermatogenesis.

The first major departure from this scheme probably occurred in the line leading to the dragonflies (Odonata). According to Oksala (54) the meiotic divisions in this order are "post-reductional," i.e., the centromeres divide in the first division but not in the second (this being the reverse of the usual behavior), and the chromatids are held together between the two meiotic divisions by residual "half chiasmata" connecting their ends. Oksala considers that dragonfly chromosomes are monocentric but some other authors believe them to possess multiple or "diffuse" centromeres [Battaglia & Boyes (2)]. It is at any rate clear that the meiotic divisions are anomalous in both male and female Odonata.

Somewhat similar anomalies of meiosis occur in some of the Homoptera and Heteroptera, although the interpretation is not as clear as one might wish. Following Schrader (61) and Hughes-Schrader & Ris (35) it has generally been believed that the chromosomes of these insect orders either have a diffuse centromere activity or are polycentric. However, Mendes (44) has claimed that the chromosomes of a species of *Dysdercus* (Pyrrhocoridae) each have a single localized centromere, and Dutt (19) has made a similar claim for a species of pentatomid. Thus although "diffuse centromere activity" is probably of rather general occurrence in the Heteroptera and Homoptera, exceptions may occur. Experimental studies on members of groups suspected or alleged to lack localized centromeres are badly needed.

As far as the meiotic mechanism is concerned, Helenius (28) concludes that it is of the "post-reductional" type in both spermatogenesis and oögenesis of scale insects and aphids and in the oögenesis of some Heteroptera (Lygaeidae). The usual "pre-reductional" type of meiosis occurs in the spermatogenesis of some Homoptera (Auchenorrhyncha) and in the spermatogenesis of most species of Heteroptera (although the sex chromosomes may behave "post-reductionally") [see White (82, fig. 97)].

The extraordinary modifications of the meiotic divisions which occur in some of the scale insects have been described by Hughes-Schrader (31). In the tribe Iceryini the male is a haploid and arises from an unfertilized egg. In some species (e.g., *Icerya purchasi* Maskell) the apparent female is actually a hermaphrodite, with an ovotestis whose ovarian part is diploid, while the testicular portion is haploid. The Aleurodidae also have haploid males [Schrader (60)].

The chromosome cycle of the Anoplura and Mallophaga is of a very characteristic and unique type [Hindle & Pontecorvo (30); Scholl (58)]. The reduction to the haploid number in the male takes place in the primary spermatogonia, so that the secondary spermatogonia and primary spermatocytes are already haploid. At the stage when meiosis usually occurs, there is only a single division into two cells of very unequal size, the larger of which becomes a sperm. The fact that the Anoplura and Mallophaga share these peculiar features is evidence of their close relationship. The chromosomes in

both cases probably have diffuse or multiple centromeres [Bayreuther (4)].

The holometabolous orders of insects seem to belong to three main stocks: (*a*) the Hymenoptera, (*b*) the Coleoptera and Strepsiptera, and (*c*) the remaining orders.

The outstanding feature of hymenopteran cytology is the haploid condition of the males [general reviews by Whiting (87); White (82)]. Although diploid males can be obtained experimentally in *Habrobracon*, all naturally-occurring hymenopteran males seem to be genetically haploid and arise from unfertilized eggs. They have only one effective maturation division in their spermatogenesis which is a simple mitosis, without reduction of chromosome number. In all Hymenoptera except the bees (Apidae) this division produces two equivalent spermatids, but in the latter it gives rise to one functional spermatid and a minute cell which degenerates.

As a consequence of these cytological facts all genes in the Hymenoptera behave as if sex-linked. The genetics of natural populations has hardly been studied at all in the Hymenoptera but must be expected to follow somewhat different principles to those which govern populations of insects that have both sexes diploid.

There is no definite evidence as to how the haplodiploid genetic system of the Hymenoptera arose (probably in Permian times). Possibly it originated in a species that already had most of its genes X-linked. At any rate a monophyletic origin seems probable. Thelytoky (a form of reproduction in which females produce daughters by diploid parthenogenesis) has partially or completely replaced haplo-diploidy in some hymenopteran species.

The Coleoptera include one peculiar species (*Micromalthus debilis* Leconte) which likewise has haploid males [Scott (63)]. But no direct relationship to the Hymenoptera seems possible, and it is likely that male haploidy has arisen five times in the Insecta: in the Aleurodidae, iceryine coccids, Hymenoptera, *Micromalthus*, and probably in the Thysanoptera where there is circumstantial genetic evidence of male haploidy, but no cytological confirmation as yet.

The remaining Coleoptera (apart from a few parthenogenetic species of weevils) have a very orthodox chromosome cycle with monocentric chromosomes and a normal meiosis in both sexes. The males are heterogametic, and in many species there is a peculiar type of XY chromosome pair which forms a "parachute-shaped" bivalent at meiosis, apparently because the terminal regions of the X are homologous to those of the Y, the middle regions being different [Smith (64, 65, 66)]. This type of sex chromosome pair is not known in other orders of insects. Various other types of sex chromosome mechanisms are widespread in the Coleoptera but are probably derivative from the "parachute" type. In the tiger beetles (*Cicindela* spp.) and the tenebrionid genus *Blaps* there are complex sex chromosome mechanisms involving several kinds of X's (X₁, X₂ · · ·) [Smith & Edgar (69); Guénin (26)]. In *Blaps polychresta* Forskål there are as many as 12 different X's and 6 kinds of Y's [Guénin (27)].

Many species of the weevil genus *Brachyrhinus* (*Otiorhynchus*) reproduce parthenogenetically and lack males. Most of them are polypoid (triploid, tetraploid, and pentaploid) [Suomalainen (70)]. The fact that these forms have no meiosis has permitted them to develop these polyploid karyotypes which undoubtedly allow a kind of heterosis. But the advantage so gained is likely to be a short-term one for which they have sacrificed the possibility of genetic recombination upon which all long-range adaptive radiation of populations and species depends.

The Coleoptera are, in general, rather conservative in regard to variation in chromosome number, and we do not find the wide range of numbers within single genera and families which are rather characteristic of the Lepidoptera, for example. Exceptions occur, however. Thus the haploid numbers of the Curculionidae are known to range from 9 to 24 [Takenouchi (72)] and those of the Silphidae from 7 to 20 [Smith (66)].

The orders Mecoptera, Neuroptera, Diptera, Siphonaptera, Trichoptera, and Lepidoptera are often regarded as the so-called panorpoid complex. The three genera of Mecoptera that have been studied cytologically show an orthodox type of genetic system with both sexes diploid, the males being heterogametic [Naville & de Beaumont (51); Cooper (13); Matthey (43)]. Cytology provides evidence of the close relationship between the Trichoptera and Lepidoptera, these being the only orders of insects to show female heterogamety. At some stage in the evolution of this stock from their mecopteroid ancestors (and presumably at a very early stage) the original mechanism of male heterogamety must have been replaced by female heterogamety. It is hardly possible to suggest the precise way in which this was accomplished, but it seems more likely that it occurred in a single species (from which all Trichoptera and Lepidoptera are descended) than that it arose independently in several lineages. At any rate, we do not find in insects that species with female heterogamety occur sporadically in groups with male heterogamety, or vice versa, so that this transformation is probably only possible under very special circumstances. It is true that a change from male to female heterogamety has been produced in genetical experiments with the fish *Lebistes* and that in some other fish species both male and female heterogamety co-exist in different races of the same species. But these fishes have a very elementary type of sex determining mechanism, the X and Y differing only in a very short region, perhaps a single genetic locus, whereas insects in general have highly developed sex chromosome mechanisms. Thus these transformations in fishes hardly help us to understand what happened when female heterogamety became established in one branch of the panorpoid complex.

The status of centromeres in the Lepidoptera and Trichoptera is very uncertain. It has been suggested that they have "diffuse" centromeres, but the evidence is indirect. In all probability meiosis is of the "post-reductional" type in oögenesis but normal in the males [Suomalainen (71)]. Chiasmata are present in both oögenesis and spermatogenesis, but in the

females they are terminally localized, so that they must be rather ineffective genetically.

In contrast to the Lepidoptera, the Diptera all seem to have strictly monocentric chromosomes, and there is no tendency to "post-reductional" meiosis. Of all the insect orders the Diptera have, in general, the lowest chromosome numbers, the great majority of the species having from three to six pairs. No member of the order is known to exceed ten pairs.

In the lower Diptera (Nematocera) we find a bewildering variety of meiotic mechanisms, chromosome cycles, and methods of sex determination. Some Tipuloidea have chiasmata in the male and a pair of cytologically distinguishable sex chromosomes. Others seem to have lost one or other of these features [Bayreuther (5)]. These two tendencies (i.e., to loss of chiasmata in the male and to disappearance of the visibly distinct X and Y) seem to have manifested themselves in several different phyletic lineages in the Diptera. Thus chiasmata are lacking in the males of the Phryneidae, Bibionidae, Scatopsidae, Thaumaleidae, Blepharoceridae, and Mycetophilidae and apparently also in most of the "higher" Diptera (Brachycera), including *Drosophila*. However, *Aphiochaeta xanthina* Speiser (Phoridae) is stated to have chiasmata in the male [Barigozzi & Petrella (1)]. The lack of chiasmata in the males of so many Diptera is associated with a special type of pairing which holds the chromosomes together throughout their length until they are torn apart at the anaphase of the first meiotic division.

In the two families, Sciaridae and Cecidomyidae, highly peculiar chromosome cycles have been developed. In both the sex of the individual depends on the elimination of one or more X chromosomes from the somatic nuclei during early embryology. Furthermore, in both families there are unipolar first meiotic divisions in the males, with particular chromosomes passing invariably to the pole, the others being left behind. In the Cecidomyidae there are always a large number of "extra" chromosomes which are present in the cells of the germ line but get eliminated from the soma during the early cleavage divisions [White (79)]. In the Sciaridae the extra chromosomes are few in number and absent altogether in some species [Metz (46)].

These highly anomalous chromosome cycles presumably developed from the much more orthodox type of genetic system found in the Mycetophilidae. The modifications have been more profound in the Cecidomyidae, which have bizarre types of meiosis in both sexes; whereas in *Sciara* the female meiosis is regular.

The Culicidae, Chironomidae, Simuliidae, and Psychodidae have a normal meiosis with chiasmata in the male but, in general, lack morphologically identifiable sex chromosomes. However, in certain species of *Chironomus* the existence of X and Y chromosomes has been proven by studying partially or completely sex-linked inversion sequences [Beermann (6)].

Cytotaxonomic Differences Between Species

Serious analytical study of cytological differences between species has only proved possible in groups where the chromosomes are not too numer-

ous. Following on the early pioneer work of Metz (45), Patterson & Stone (56) have made an outstanding analysis of the cytotaxonomic differences between species of *Drosophila*, based on the somatic metaphase configurations of approximately 200 species, supplemented by studies of the salivary gland chromosomes. Later work on additional species [Clayton & Ward (12)] has not materially affected their conclusions.

Apart from paracentric inversions, which do not change the metaphase shape of the chromosomes (and of which some thousands or tens of thousands must have occurred in the phylogeny of the genus *Drosophila*), Patterson & Stone consider that fusions, pericentric inversions, and additions of heterochromatin have contributed to the cytotaxonomic variation. They postulate 54 fusions (34 between autosomes of similar length, 6 between a long and a very short autosome, 15 X-autosome fusions, and 1 Y-autosome), 30 pericentric inversions, and 32 additions of heterochromatin. It is probable that this interpretation, based on the minimum number of rearrangements needed to explain the facts, is actually an over-simplified one, the number of cytological changes having been considerably greater. The analysis is founded on the assumption that the original karyotype of the genus consisted of five long pairs of acrocentric chromosomes and one pair of short acrocentrics ("dots"). Patterson & Stone's 54 different centric fusions are between the various members of this chromosome set, to give different kinds of metacentrics. Although the authors discuss the theoretical possibility of dissociation of a metacentric element into two acrocentrics (a Y or a supernumerary chromosome supplying the additional centromere and two telomeres which are required, if the dissociation is to be viable), they do not believe that any change of this kind has been established in *Drosophila*. Obviously, the difficulty is one of deciding in each case whether two acrocentrics have given rise to a metacentric or vice versa.

The differences between the chromosome sets of *Drosophila* species are, in general, rather considerable. Thus members of the same species group often have very dissimilar karyotypes. In the section Calyptratae of the "higher" Diptera the great majority of the species have five pairs of metacentric autosomes and an XY pair of rather variable dimensions [Boyes & Wilkes (9); Boyes (8)].

Fusions between sex chromosomes and autosomes are particularly interesting from a theoretical standpoint, since they result in genes which were formerly autosomal becoming sex-linked. The general theory of such transformations has been discussed elsewhere [White (83)]. Fusions between an acrocentric X and an acrocentric autosome will convert a previously XO sex chromosome mechanism into an XY one, the unfused homologue becoming in effect a Y. Various other types of fusions will give rise to the multiple sex chromosome mechanisms (X_1X_2Y, XY_1Y_2, etc.) which are now known in a considerable number of insects such as mantids [White (77); Hughes-Schrader (32, 34)], fleas [Bayreuther (3)], crickets [Claus (11)], grasshoppers [White (81)], and higher Diptera [Dobzhansky (18); Patterson & Stone (56); Boyes (7)].

Not all multiple sex chromosome mechanisms seem, however, to have arisen by incorporation of autosomes; some of those known in the Heteroptera and Homoptera at any rate have more probably come about through "dissociation" of the original X or Y into several components (the required extra telomeres, and possibly centromeres as well, being supplied by other X's, Y's or supernumerary elements).

Although in many groups there have been numerous incorporations of previously autosomal chromatin in sex chromosomes, hardly any instances of the reverse type of change are known. In the case of *Drosophila ananassae* Doleschall and possibly some related species, however, the proximal segment of the X, which is heterochromatic and bears the nucleolus, has been translocated to one of the autosomes [Kaufmann (36); Kikkawa (37)].

It is not entirely clear why certain groups of insects should show much more striking and extensive cytotaxonomic differences between species than other groups having essentially the same type of population dynamics. Thus the true short-horned grasshoppers (family Acrididae) show great cytotaxonomic stability, the overwhelming majority of the species having $2n \, \male = 23$ acrocentrics (although in the subfamilies Pamphaginae and Pyrgomorphinae $2n \, \male = 19$). Only in a few genera have metacentric chromosomes established themselves, either as a result of fusions or by pericentric inversion.

In the grasshopper family Eumastacidae, however, there is much less cytotaxonomic stability. In approximately 74 species of the Australian subfamily Morabinae $2n \, \male$ ranges from 21 down to 13, and the details of the karyotype vary greatly from species to species, even where these are barely distinguishable on external morphology [White (85)]. About 70 of the forms studied have XO males (undoubtedly the original condition for the group), but 7 have XY sex chromosome mechanisms and 8 have X_1X_2Y ones, as a result of the incorporation of various autosomal pairs in the sex chromosome system. Probably XY mechanisms have arisen independently six times in this group and on four separate occasions have given rise to X_1X_2Y mechanisms. One group of very closely related species in central New South Wales includes two XY ones and two X_1X_2Y ones, the precise shapes of the sex chromosomes differing in a manner which proves that various kinds of structural changes (pericentric inversions, deletions in the Y, and possibly duplications in the X's) have occurred subsequent to the original fusions. These species, all as yet undescribed, are allopatric and would almost certainly have been regarded as races or subspecies in the absence of the cytological evidence. Hardly any copulations between them were obtained when members of the different populations were caged together, i.e., they behaved biologically as species, not as races. At least 10 species of Morabinae have their populations regularly polymorphic for chromosomal rearrangements (in most cases pericentric inversions), and several more possess supernumerary chromosomes in some at least of their populations. The "ancestral" chromosome set of the Morabinae probably

consisted of an X and eight pairs of autosomes (a large metacentric, a smaller metacentric, and six small pairs). If this interpretation is correct, the chromosome numbers below 2n $\male$ = 17 are attributable to fusions, while in species with 2n $\male$ = 19 or 21 one or two "dissociations" of the metacentrics would have taken place. Evidence for the reality of such dissociations is, in fact, much stronger in this group than in *Drosophila*, and in one case a critical experimental proof of the dissociation hypothesis has been obtained [White (85)].

The Morabinae are apterous insects of low mobility and specialized ecological requirements, so that the numerous species are mostly restricted to quite small areas. It may be that their population structure has been particularly conducive to the development of cytological polymorphism and hence of cytotaxonomic differences. But this explanation is hardly sufficient, since there are many species and genera of Acrididae whose motility is no greater than that of the Morabinae. Actually, it is quite possible that cytotaxonomic differences of a rather inconspicuous kind, namely alterations in the extent and distribution of heterochromatic segments, may be very frequent in the Acrididae. Thus, although many large genera have an apparently uniform karyotype of 23 acrocentric chromosomes in the male, there are almost always slight differences in the relative lengths of the various chromosome pairs. Many species of Acrididae are regularly polymorphic in respect of the extent of heterochromatic segments. And all types of cytotaxonomic differences which occur in the Morabinae are also known in the Acrididae, although they are less common. For example, fusions between autosomes and the X, changing the sex chromosome mechanism from XO in the male to XY, are known in at least 21 genera of Acrididae, out of several hundred that have been examined [Helwig (29); King (38); White (83); Saez (57)]. The North American genus, *Paratylotropidia*, even includes one XY species, with an X-autosome fusion, and two X_1X_2Y species, in which an additional Y-autosome fusion has occurred [White (81)].

The acridid grasshopper whose karyotype has been most profoundly modified is an unnamed species of the South American genus *Dichroplus* with 2n $\male$ = 8 [Saez (57)]. The chromosome set of this species has probably acquired no less than 8 fusions and 6 pericentric inversions bringing it down from 12 chromosome pairs to 4. Other species of the genus either have 2n $\male$ = 23 or have acquired from one to three fusions and (in one species only) two pericentric inversions. It is difficult to imagine what particular feature of the population genetics of the 8-chromosome species has favored the occurrence of so many fusions and pericentric inversions, each of which must presumably have formed the basis of a mechanism of adaptive heterosis in the past (otherwise it is difficult to see how they could have become established and spread through the species). Comparable cases are, of course, known in other groups, but this is a particularly striking one, since it is in a group characterized in general by cytotaxonomic stability.

It seems quite unlikely that relative cytotaxonomic stability in a group

such as the Acrididae is a result of the chromosomes having a low rate of spontaneous breakage, since in all organisms the number of spontaneous rearrangements that occur is probably vastly in excess of the number that can succeed in establishing themselves.

The recognition of sibling species, i.e., forms barely or not at all distinguishable in external morphology, is greatly facilitated in certain families and genera of the Diptera by the existence of the giant salivary gland or polytene chromosomes in which changes of gene sequence which would be undetectable in most groups of organisms are readily observable. The separation, as a result of cytogenetic study, of the species *Drosphila persimilis* Dobzhansky from *Drosophila pseudoobscura* Frolova was opposed by some at first, since the two could not be distinguished by a museum taxonomist. But more recently differences between the male genitalia of the two species have been found.

In the *bocainensis* subgroup of the *willistoni* group of *Drosophila*, Carson (10) has distinguished three sibling species, two of which are indistinguishable morphologically but differ with respect to 13–26 inversions. These two forms are sympatric, but hybridization apparently does not occur in nature (since the inversions do not "leak" from one species to the other), although vigorous and fertile F_1 hybrids can readily be obtained in the laboratory.

In the case of the sibling species of the *Anopheles maculipennis* group (generally regarded as races but clearly species in the biological sense and in some instances distinguishable by visible characters of the eggs and wing scales as well as by ecological characteristics) Frizzi (22, 23) has shown by a study of the salivary gland chromosomes that *typicus* Hackett & Missiroli differs from *atroparvus* Thiel by a large inversion. *Elutus* Edwards differs from *atroparvus* by a different inversion. *Messeae* Falleroni has the same large inversion as *typicus* (all these inversions are present in the homozygous state). But there are two "races" of *messeae* which differ in respect of a complex rearrangement in the third chromosome. A few hybrids between these two races were collected in nature, so that the biological isolation between them is incomplete [Frizzi (24)].

Relatively few instances of sibling species are known in grasshoppers. In one very striking case, however, chromosomal study has led to the discovery of a pair of sibling species. The Australian *Austroicetes pusilla* (Walker) was earlier regarded as having an "inland race" which apparently intergraded or hybridized with other "races" where the ranges overlapped. Recent cytological work has shown that this "inland race" is actually an entirely distinct species whose phenotype (when one considers the whole range of geographic variation and polymorphism of the two forms) overlaps with that of *pusilla* [White & Key (86)]. Thus in some areas individuals of the inland species can be found which look like *pusilla* and in other areas individuals of *pusilla* are phenotypically like the inland species. But the two differ in no less than seven cytological characters (chromosome number, shape of the X chromosome, presence of a pericentric inversion in the homo-

zygous state in one species, size of the smallest chromosome pair, chiasma frequency and type of chiasma-localization, and the fact that one species is polymorphic for several chromosomal rearrangements while the other is cytologically monomorphic). Hence, in spite of the special type of phenotypic overlap, which looked very much like evidence of introgressive hybridization before the chromosomal study disproved this interpretation, these two species are certainly not *in statu nascendi* but must have diverged completely at a relatively remote period. Chromosome cytology does not seem to have provided any evidence in favor of introgressive hybridization having occurred in insects.

In the crickets and mole crickets there are quite a number of instances in which classical species seem to be subdivisible into sibling forms (species or races) which differ cytologically. Thus *Gryllotalpa gryllotalpa* Linnaeus seems to include no less than seven forms with the chromosome numbers (2n σ) 12, 14, 15, 17, 18, 19, 23 [references in White (82)]. How many of these are full species in the modern, biological sense is uncertain. In the cricket *Loxoblemmus arietulus* Saussure, Ohmachi & Ueshima (53) distinguish three sibling species which they call by the Japanese names Tanbo-okame, Mori-okame, and Hara-okame. The chromosome numbers range from 2n σ = 13 (X+4 pairs of metacentrics+2 pairs of acrocentrics) to 17 (X+1 pair of metacentrics+7 pairs of acrocentrics). However, at one locality there are two populations of Mori-okame which differ cytologically. Since no hetero-zygotes were encountered, we suspect that Mori-okame itself consists of two sibling species which do not interbreed in nature. Similarly, at another local-ity there seem to be two cytologically distinct populations of Hara-okame which do not interbreed. Thus "*L. arietulus*" in Japan may be a complex of as many as five species. Cytologically, at least one centric fusion, two pericentric inversions, and (probably) one dissociation of a metacentric into two acrocentrics seem to have occurred. The "species" *Gryllulus mitratus* Burmeister likewise seems to consist of three sibling species whose chromo-some complements are different [Ohmachi & Matsuura (52)].

The general effect of cytological studies on insect taxonomy will undoubt-edly be to increase still further the number of instances of sibling species barely or not at all distinguishable on external characters. Such a tendency may be deplored by conservatively minded taxonomists. But we cannot dis-regard the evidence that it is the "siblings" rather than the aggregates of siblings which are the essential entities in the biological sense, i.e., the re-productive units. And the evidence of the chromosomes is in excellent gen-eral agreement with that from other fields such as physiology and ethology.

It is still an open question whether any pair of species would be cytolog-ically indistinguishable, if we were able to make accurate and detailed chromosome maps such as are only possible in a few Diptera. However, in *Drosophila* there is at least one case of very closely related species which do not seem to differ at all in gene sequence. This is the *mulleri-aldrichi-wheeleri* trio [Wasserman (75)]. There can hardly be any doubt that these are full

species and that there are no large inversions or other chromosomal differences between them, but the possibility that some minute chromosomal differences are present has not yet been ruled out. Most sibling species in *Drosophila*, even if indistinguishable in external appearance as in the *bocainensis* complex [Carson (10)] differ in gene sequence. Thus it may be that every species of animal is chromosomally unique, but at present the *mulleri* trio seem to constitute evidence against this view. In groups without giant salivary chromosomes we must expect to find that many sibling species are cytologically indistinguishable by present day methods. However, it is now clear that in the majority of cases the process of speciation involves the acquisition of karyotypes which are cytologically different. This is presumably because it is frequently associated with the development of different systems of chromosomal polymorphism in allopatric populations constituting "incipient species" [Wallace (74); White (83)].

In groups with haploid males the chromosome sets seem, on the whole, to be rather uniform from species to species. The ants are a group with extreme uniformity; 19 species belonging to 6 subfamilies all showed the same number (n = 5) of small chromosomes, approximately equal in size [Wheldon & Haskins (76)]. On the other hand cytotaxonomic variation certainly occurs in some groups of Hymenoptera. Thus in five species of the wasp genus *Polistes* the haploid number ranges from 6 to 21 [Machida (42); Pardi (55)]. In the Hymenoptera, of course, chromomosomal polymorphism can only give rise to heterosis in the females, i.e., it must play the same role as rearrangements in the X-chromosomes of *Drosophila* and grasshoppers do.

In order to understand how differences in chromosome number have come about in the course of evolution we need to study instances where different chromosome numbers co-exist within the same species. Several different kinds of such intraspecific variation in chromosome number are known.

Populations regularly polymorphic for the presence and absence of fusions, i.e., with many individuals heterozygous and showing a trivalent composed of two acrocentric chromosomes paired with a metacentric one at meiosis, have been described for the mantid, *Ameles heldreichi* Brunner [Wahrman (73)] and the bark weevil, *Pissodes approximatus* Hopkins [Smith (67)]. In both instances the polymorphism exists in most or all populations of the species, so that it is presumably adaptive, probably because the heterozygotes are heterotic.

A very different situation exists in the grasshoppers, *Trimerotropis sparsa* Thomas, from the Great Basin [White (80)] and in the Australian *Moraba scurra* Rehn[White (84)], in both of which there are geographic races with different chromosome numbers. In each case one race has a fusion which the other lacks, but in *T. sparsa* the "unfused" condition probably gave rise to the fused one, while in *M. scurra* there is experimental evidence that the reverse process occurred [White (85)]. Strictly speaking, we should call individuals of *T. sparsa* with a trivalent at meiosis fusion-heterozygotes, the corresponding individuals of *M. scurra* being dissociation-heterozygotes. In

T. sparsa there seems to be a narrow zone of overlap between the two races across western Colorado and eastern Utah, within which some colonies are polymorphic for the fusion, so that a few heterozygotes occur. In *M. scurra* the two races are contiguous over a distance of at least 150 miles, but no colonies regularly polymorphic for the dissociation have been found as yet, although occasional dissociation-heterozygotes (about one in five hundred individuals) have been encountered in populations otherwise monomorphic for the absence of the dissociation. It is probable that the absence of a narrow zone of overlap containing polymorphic populations is a recent development, following widespread deterioration of the habitat of the species as a result of grazing by sheep in the past hundred years.

In those cases where fusion, or dissociation, heterozygosity is restricted to a narrow zone or is extremely rare over a larger area there is no reason to believe that it is heterotic (i.e., in *T. sparsa* and *M. scurra* such heterozygotes may have a lower adaptive value than either homozygous type). The races of these two forms may be incipient species, but no genetic isolating mechanisms seem to have been developed as yet.

Fusion heterozygosity also occurs in some populations of the grasshoppers, *Hesperotettix viridis* (Thomas) and *Circotettix undulatus* (Thomas) [Evans (20)], but these cases have not been studied sufficiently for any firm conclusions to be drawn. In the Coccinellid beetle, *Chilocorus stigma* Say, some individuals are heterozygous for three different fusions [Smith (68)].

There is very little evidence that supernumerary chromosomes have been directly responsible for evolutionary increases in chromosome number, i.e., that they can eventually become stabilised and permanent members of the chromosome set. However, they may have contributed centromeres and telomeres in cases where metacentric chromosomes have undergone "dissociation" into acrocentrics. True supernumerary chromosomes are elements which are usually in large part heterochromatic and which are not homologous, or only partly homologous with the members of the regular chromosome set. Their mode of inheritance is frequently irregular. They vary in number from none to several in the individuals comprising the populations, and local colonies of the species usually differ in the mean number of supernumeraries per individual. Closely related forms may differ in the presence or absence of supernumerary chromosomes. Thus in most populations of the Australian grasshopper, *Atractomorpha crenaticeps australis* Rehn, supernumeraries are very numerous, so that individuals lacking them are a small minority; while in *A. crenaticeps crenaticeps* (Blanchard) supernumeraries do not seem to occur [White (85)].

Since in some insects with "diffuse centromere activity" it has been shown experimentally that all chromosome fragments resulting from irradiation will attach themselves to the spindles in subsequent divisions, it has seemed to some cytologists that simple chromosome fragmentation should be possible in such groups and that great cytotaxonomic variation in chromosome numbers would result. This viewpoint, however, neglects the telo-

meres, and it seems likely that the processes of chromosomal evolution are not widely different in such groups. In fact, some families of the Heteroptera, such as the Corixidae and Pentatomidae, show great constancy in chromosome number, while others such as the Lygaeidae, Coreidae, Miridae, Reduviidae, and Belostomatidae, show a greater range.

In certain genera of Lepidoptera and Trichoptera (groups which may exhibit "diffuse centromere activity," this is not certain) there is an extremely wide range of chromosome numbers, e.g., from $n = 8$ to $n = 40$ in closely related species of *Erebia* [Lorković (40, 41); Federley (21); De Lesse (15)] and from $n = 23$ to $n = 191$ (the highest known chromosome number in animals) in the lycaenid genus *Lysandra* [Lorković (40); De Lesse (16, 17)]. It was formerly suggested that the higher numbers in such cases were attributable to polyploidy, but there are strong arguments against this view [White (78)]. A detailed interpretation is hardly possible in view of our ignorance of the status of centromeres in this group and because there is too little information about the relative sizes of the chromosomes, extent of heterochromatic segments, etc. However, we are inclined to believe that orthodox explanations based on "fusions" and "dissociations" will suffice to explain these cases. But it is certainly remarkable (if true) that the Spanish *Lysandra nivescens* (Keferstein) has undergone no less than 168 dissociations in the course of its phylogeny as a member of this genus (raising the chromosome number from $n = 23$ to $n = 191$).

Certain types of cytotaxonomic differences between species which are not, or only doubtfully, a result of structural rearrangements, may be briefly mentioned. In some groups one can distinguish between species which have long slender chromosomes at metaphase and others which have much thicker, more contracted chromosomes. Thus chromosome elements which are undoubtedly homologous from species to species may look quite different.

The amount of deoxyribonucleic acid (DNA) per chromosome set is probably a constant for each species. But this quantity may vary considerably even between closely related species. Thus Hughes-Schrader (33) finds that out of four species of the mantid genus, *Liturgousa*, two have about one and one half times as much DNA as the other two. It is uncertain whether differences of this kind are attributable to variation in the size of heterochromatic segments or whether they are associated with differences in the metaphase thickness of the chromosomes. In the pentatomid genus *Thyanta*, however, six species show great uniformity in their DNA values [Schrader & Hughes-Schrader (62)].

Our discussion of cytotaxonomic differences has been concerned mainly with "major" rearrangments easily detectable under the microscope. However, it is probable that "minute" changes involving duplication or deletion of single genetic loci or small groups of loci have been at least equally significant in genetic evolution. In the salivary chromosomes of *Drosophila* and other Diptera there are many so-called "doublets," apparently consisting of two adjacent bands of identical appearance. It is now fairly clear that a

relationship exists between such doublets and the pseudoallelic complexes studied genetically by Lewis (39), Green (25), and others. It has generally been assumed that these doublets have arisen by evolutionary duplication, followed by functional divergence. If so, about one third of the genetic loci of *Drosophila melanogaster* Meigen have undergone this kind of evolutionary duplication. But the "evolutionary duplication" interpretation is still an unproven hypothesis, and alternative possibilities certainly exist.

For understanding the relationships of the intraspecific categories of races, microgeographic races, and local populations, cytology is likely to become increasingly important but only in those species which exhibit some kind of cytological polymorphism. A number of species of *Drosophila* have been rather thoroughly studied from this standpoint [see Da Cunha (14) for a general review], and a beginning has been made with such grasshopper species as *Trimerotropis sparsa* and *Moraba scurra*, both of which have numerous pericentric rearrangements (probably in all cases inversions) in addition to the chromosome number polymorphism referred to above. A particularly interesting application of this approach is likely to be the analysis of geographic variation in some parthenogenetic species of Diptera by the salivary chromosome technique [Scholl (59)].

SUGGESTIONS FOR FUTURE WORK

We have tried to show the significance of cytogenetics at different levels of systematics, from the study of the "higher categories" down through the species level to the investigation of races and local populations. Cytogenetics is not an automatic solution to all systematic problems, neither is it a final court of appeal in all difficult taxonomic situations. But in suitable cases it can provide critical evidence of a unique kind. It thus seems desirable that serious systematists should have some understanding of the methodology and procedure of the cytologist. And it is surely to be hoped that all really detailed and extensive systematic monographs should include basic cytological information. In some instances a brief statement such as "All species of this group have $2n = 12$; the chromosomes are small and dot-like both in spermatogonial and meiotic divisions" would suffice, while in other cases the cytological information would play a highly important role in the systematic treatment. Modern techniques (using squashes rather than sectioned material) have made chromosome work much less laborious than in the past. However, in the case of material collected on expeditions, or under conditions where no laboratory facilities are available, it is still necessary to fix material which will later be embedded and sectioned. For this purpose it is usually sufficient to remove the testes from the insect and fix them in Navashin's fluid, in which they may be stored for up to several weeks. The most convenient method is to prepare two sets of shell vials before setting out on an expedition, one set containing 4 cc. of a 1:8 mixture of glacial acetic acid and 1 per cent chromic acid, the other containing 2 cc. of formalin. The vials should be provided with plastic stoppers. Immediately

before each fixation the contents of a "formalin vial" and a "chrom-acetic vial" should be mixed by pouring from one to the other several times. A series of printed or typewritten numbers, in duplicate, should be used, one number being placed in the vial containing the fixed testis and the duplicate number on the pinned specimen. A pair of fine scissors and forceps (watch-maker's forceps for small insects) are the only instruments required, and the fact that insect testes usually have a pigmented envelope makes dissection surprisingly easy, even under field conditions. In order to obtain the best results, the testes should always be removed by vivisection and not from insects already dead. In some groups of insects the spermatogenesis is completed before the imaginal stage is reached, so that it is necessary to obtain the testes from pupae or last-instar nymphs.

Before collecting extensive cytological material of a particular group it would seem desirable to find out what is already known of its cytology, since further cytological work on a few groups (such as the ants) is likely to be rather unrewarding.

Cytological studies of larval salivary gland chromosomes or of somatic chromosomes in squashes of larval brains are still only practicable where adequate laboratory facilities are available.

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THE TAXONOMIC SIGNIFICANCE OF THE CHARACTERS OF IMMATURE INSECTS[1]

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In most insects of economic importance the stage causing damage is the immature stage alone; at any rate, it does not affect man less than the adult. As a consequence of juvenile mortality the number of larval insects in any insect population is necessarily much higher than that of adults, and in many groups the duration of the immature stages much surpasses that of adult life. In marked contrast with this prevalence and importance of the early stages, their taxonomic characters are to a great extent unknown. For instance, the relatively small and especially well-explored beetle fauna of the British Isles does not contain a middle-sized or large family of which all species are known in the larval stage, let alone as eggs and pupae, and there are several families of two score species in which not more than two or three species have been described adequately (e.g., Trichopterygidae, Pselaphidae). It will clearly take more than a century to obtain and describe all the unknown stages of insects even of a smaller European country, but by classifying the known forms we are able even now to draw conclusions upon the characters of unknown genera and species and thus to "identify" these more or less exactly. As insect larvae are usually regarded as caenogenetic (secondary) developmental stages, their importance for phylogenetic conclusions and classification is often considered to be slight. The degree to which their ontogeny recapitulates phylogeny, however, is precisely the scale by which the taxonomic significance of their characters is measured, so that their importance for taxonomy depends largely on the measure in which they are phyletic. The knowledge of the larvae as the only fully active and completely "organized" pre-imaginal form is of greater practical and theoretical importance for taxonomy and phylogeny and much more advanced, although that of eggs and pupae also has significance in many cases. The larvae will therefore take up by far the greater part of this review.

THEORETICAL FOUNDATION

Larva.—If insect larvae are often called caenogenetic or secondary larvae, this applies especially to the Holometabola, which comprise five-sixths of the described recent insects [Peterson (110)]. It is obvious, for instance, that a stagbeetle cannot at any period of its phylogenetic evolution have been

similar to a stagbeetle larva, whereas a springtail or silverfish and its larva must always have resembled each other greatly. In fact, at some very early time of their phylogeny both the stagbeetle and its larva must have been somewhat similar to those insects. As the typical larva, thus, does not represent an earlier phylogenetic stage of the winged insect, the larval characters cannot be regarded as a general rule to be of overriding importance for the taxonomy. This applies to a greater or smaller extent to all insect orders, as the larvae have acquired characters which the ancestors cannot have had as adults. Moreover, to the general zoologist all insect larvae are caenogenetic, as none of them "represent ancestral forms which preceded the earliest insects. Therefore, all that insect larvae can teach us can only concern the precincts of this class" [Korschelt & Heider (88)]. However, in Collembola, Thysanura, and, somewhat more, Protura the larva deviates only to a slight extent from the primitive form. Even in the groups centering round Orthoptera, Psocoptera (=Copeognatha), and Hemiptera the acquired characters of the larva are only moderately pronounced and often less marked than those of the adult. On the other hand in the aquatic orders of Hemimetabola strong secondary adaptations have taken place, which are in some ways comparable with those in Holometabola, where again differences in biology are mainly responsible for the acquired features. Entomologists have, therefore, divided insect larvae into forms with direct metamorphosis or imaginipetal [Börner (15)] larvae, often called nymphs (6, 19, 79), and those with an indirect or complete metamorphosis, caenogenetic [Comstock (35)] or imaginifugal [Börner (15)] larvae or "larvae" (6, 19, 79) in the restricted sense. The term caenogenetic is here given a different meaning, referring to the phylogeny of the orders within the class Insecta only, and, restricted in this way, the term "phyletic" can be applied to the primitive types of all insect larvae in so far as they are all characterized by the primary absence of wings [Börner (15)]. Other authors, emphasizing the acquired characters of the aquatic Hemimetabola etc., distinguish primary, secondary, and tertiary larvae [Hayes (67)] or nymphs, naiads (35, 108), and larvae. These divisions and even more the augmentation of the three groups Ametabola, Hemimetabola (or Heterometabola), and Holometabola to 12 or more different types of metabolous insects (15, 69) are of little practical use and serve only to underline the absence of sharp borderlines in the phenomena of natural history. Thus, the active last instar of the Hemimetabola, which is often called "nymph" in a different sense (31, 66), is connected by all external morphological transitions with the pupa of the Holometabola. In Aleurodidae, for instance, the fourth instar is at first a normal feeding stage as in other Hemimetabola (though without external wing pads), but after it has ceased feeding, the winged adult develops therein, and at last emerges from it without undergoing any moults inside the fourth instar [Weber (141)]. Female Coccidae behave like normal Hemimetabola, but the male does not feed in the third instar, and after a moult becomes a creature which is similar to the pupa of a Holometabolous insect

(but capable of movement and of secreting a new cocoon if exposed) [James (80)]. In Holometabola the pupa of the Megaloptera is able to walk about, and in some completely neotenic females of Lycinae the moult from the (whitish) pupa to the (brown) adult is vestigial (whereas the brown larva transforms into the pupa by a normal ecdysis) [Koningsberger (86); Mjö-berg (104)]. Prothetelic malformations of larvae of Holometabola also help to bridge the gap [Carpenter (28)]. Holometaboly and the "tertiary" larvae are thus connected by every transition with ametaboly and larval forms which show hardly any acquired larval characters. It is obvious from the many transitional forms that the larva of the Holometabola has not come into being only relatively recently, after the adults had already attained the distinctive characters of beetles, butterflies, or two-winged flies, but that adults and larvae have gradually developed simultaneous and concurrent specialisations in diverging directions [Börner (15); van Emden (46); Bor-radaile *et al.* (19)]. The inheritance of the separate larval and adult characters, which may be diametrically opposed (see p. 95), is made possible by the polymorphic potentialities [Wigglesworth (144)] of the insect organism (as much as for instance that of the striking differences between the genera-tions of adult gall wasps), the larvae having of course no gene-flow of their own but only that of the adults. Theoretically, larval and adult characters are thus absolutely equivalent for taxonomy [van Emden *apud* Gordon (53)].

Egg.—The characters used in classifying insect eggs are essentially those of the secondary and tertiary egg shells, chorion, and gelatinous masses etc., though Berlese (11) has attempted to group insect larvae by embryonic char-acters. The result was that very highly differentiated larvae (of Chalcidi-dae and Lepidoptera) are interpreted as the most primitive types, whereas the larvae of the Ametabola and Hemimetabola would hatch in the most adaptive condition. Crampton (36) has sharply rejected this "fantastic idea," and Hinton (74) has recently proved that the prolegs of the Lepidop-tera are not homologous with thoracic legs but secondary adaptive struc-tures and has thus destroyed what was perhaps the main support of Berlese's theory. In the various orders Seidel and his school have discovered interest-ing differences in the position of the kinetic centres etc. of embryogenesis [Krause (89)], and in Chrysomelidae they have even found generic charac-ters, for instance in the thickness and structure of the blasteme, and specific characters, for instance in the position of the blastoderm nuclei [Seidler (126)]. It remains to be seen to what extent these characters can be used to define natural taxonomic groups when greater numbers of genera and species are studied, but the application of truly embryological egg characters to classification and phylogeny is certainly a remarkable development. The structures of the chorion, on the other hand, reflect the shape of the follicular cells in the ovariole and are thus the manifestation of a character of the adult (38). Whatever is their significance for taxonomy, it must therefore be identical with that of other adult characters. To an even larger extent the same applies to the tertiary egg shells, which are formed by secretions of the

female genitalia in combination with actions based on inherited instincts of the adult.

Pupa.—Whereas the final instar of typical Hemimetabola is almost as much a larval stage as the previous stages, this is very different in the last preimaginal stage of the Holometabola, the pupa. Although, phylogenetically, the pupa is derived from, and connected by both normal and teratological (prothetelic) transitions with the last larval stage (see p. 92), it is in nearly every respect (head, mouthparts, antennae, thorax, legs, abdomen, exterior genitalia) like the adult [Hinton (73)], though limbs and appendages are shorter and the form of the various parts softened. In its basic structure the pupa thus shows "adult" features, and as far as these go, the significance of its characters is identical with that of the characters of the adult. However, the cuticle of the pupa is produced by the same hypodermis cells which secrete the preceding larval integuments, so that some relation between pupal and larval characters might be expected. This relation would be most likely where the prepupal rest is short and the larval skin shed and least likely where the latter surrounds the pupa as a protective armour during the pupal life so that the transformation can proceed at leisure (e.g., in Diptera Cyclorrhapha). Nevertheless, few direct connections between the characters of larvae and typical pupae have been proved, the most important perhaps being the presence of, somewhat differently arranged, larval ocelli behind the pupal (=adult) eye in Dytiscidae [Korschelt (87); Bertrand (12)]. In some Culicidae pupal and larval chaetotaxy are similar, and these setae have been considered homologous [Belkin (8)], but neither their number nor relative size nor position are truly identical in the two stages, so that further study will be necessary. On the other hand, pupal evolution often goes its own way, pupae in many cases having fewer functional spiracles than either larva or adult (42, 107, 131), or well-developed cerci where these are absent (42, 107) or vestigial (22) in the larva. They often have special sclerotized tubercles, teeth, and processes on the abdomen enabling them to move in or out of their cell ["Acanthopleona," Börner (17)], to suspend themselves (39, 56), to defend themselves against certain enemies (72), or to provide space for dense long hairs (102). They, therefore, have undergone a considerable diverging specialisation of their own, so that their specialised "pupal" characters have a similar importance to those of the secondary larvae. As the pupa is of more recent origin than the larva this importance should be expected to be less decisive, and what is known of pupal classification seems to support this view. On the other hand Paulian (107) considers adaptive characters as practically lacking in pupae (which view is hardly right) and believes therefore that the taxonomy of the pupae might be of greater importance than that of both adults and larvae.

Influence of adaptation.—In practice, the characters of one stage may become less important than those of another, as the influences of milieu and adaptation may modify one stage more strongly than the other [Parker (106)]. Even in terrestrial Hemimetabola, where the larvae are essentially

primary, the winged orders of adults have undergone a considerable degree of differentiation of their own. Thus, in Hemiptera Heteroptera the two series Gymnocerata and Cryptocerata can be separated as larva and adult by the same character (to which the names refer), but the rest of the classification has to be based on independent characters [Jordan (83)]. In the amphibious Hemimetabola the larvae show often much more striking adaptations than the relatively uniform adults [see figures in Schönemund (125)], although the mouth parts may allow an identical classification of larvae and adults [Frison (58)]. In larvae and adults of Holometabola, where the biology is normally very different and where at any rate the resting pupal stage with its histolysis enables a much more drastic metamorphosis to take place, structural similarities between corresponding parts of larva and adult can no longer be expected [van Emden (46)], so that the "phylogeny of the characters" cannot be derived from adult but only from larval features [Hennig (68)]. In fact, characters may be so different, that they are exchanged between larvae and adults of two genera, e.g., the stoutness of the body in the weevils *Ceuthorrhynchus* and *Calandra* (= *Sitophilus*) and the toothed and toothless mandible in the beetle genera *Malthodes* and *Malthinus* [Verhoeff (139)]. Among Holometabola strongly adaptive characters are found for instance in larvae of chafers and short-nosed weevils which live in soil, mostly feeding on roots, whilst the adults are pests of leaves and flowers; in fly maggots living in decaying matter or as parasites in other insects, whereas the adults mainly take nectar of flowers; and larvae of sawflies and butterflies and moths, which feed on leaves and show such striking convergence, whereas the adults visit flowers and show no similarity whatsoever. Larval and adult evolution are influenced so much by these differences of habitat, that their salient characters must have developed in different directions since the time when the groups in question first appeared. The importance of the characters of the immature stages can only be enhanced by such diverging trends, as these ensure that those characters offer a truly independent means for checking the soundness of an existing classification based on the adults "as though the phases were organisms independent of each other" [Hennig (68)].

Where one of the stages remains much more homogeneous than the other, genera, species-groups, and species are more readily recognised in the more highly differentiated stage. This is the case for instance in short-nosed weevils with very homogeneous larvae [van Emden (51)] and click beetles of the genus *Corymbites* (= *Ludius* of most American authors) with very uniform adults but very heterogeneous larvae. Conversely, the higher aggregates of classification are often obscured in insects with a high degree of differentiation, whereas they may be more evident in the more uniform stage, e.g., the larval stage of Lucanidae [van Emden (52)] and probably the adult in Lycinae [see figures of heterogeneous larvae by Rosenberg (119)].

Theoretically, then, the characters of the egg-chorion and egg-masses are manifestations of characters of the adult, and the basic morphological

characters of the pupa are those of the adult so that to that extent the characters of these two stages have the same significance as other adult characters. The special pupal characters and the characters of the larvae, on the other hand, have developed concurrently with, but in diverging direction from, those of the adults. As this evolution began at an early stage of the phylogeny, the significance of these characters is equivalent to that of the adult for a different reason. In any particular group, however, one of the stages may acquire a prevailing importance as a result of the special evolutionary trends of the group.

TAXONOMIC RESULTS OF THE CLASSIFICATION OF IMMATURE INSECTS

Agreement between larval and adult groupings.—It has been known for a long time that characters of immature insects very often define the same groups as are obtained by unrelated characters of the adult (Fig. 1A), and
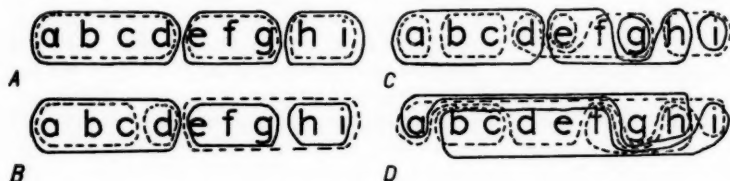
FIG. 1. Agreement between larval and adult groupings. [Modified after Hennig (68)] a to i = taxonomic groups; full lines = adult characters; dotted lines = larval characters. According to Hennig: A = complete congruence; B = spurious incongruence; C = reparable incongruence; D = irreparable incongruence. According to van Emden: A = congruence; B = similarity; C = similarity in need of correction; D = incongruence.

such groups have been termed congruent groups [Weismann (142); Lenz (93); van Emden (46)]. Groups of larvae which are not identical with the aggregates in which their adults are united are called incongruent (Fig. 1B to D) by both the former authors, whereas I have divided these into "similar" (Fig. 1B) and "incongruent" (Fig. 1C and D) groups, reserving the latter term for "quite inconsistent" groupings in the two stages. "Similar" groups, on the other hand, are those which are defined by the characters of one of the stages in a wider sense than by those of the other stage, without incorporating strange elements. Genuine incongruities exist for instance in short-nosed weevils [van Emden (51)]. They are rare and indicate that one of the systems concerned is unnatural, i.e., phylogenetically wrong [van Emden (46, 47); Hennig (68)].

Useful characters.—The available room does not allow a discussion of the type of characters used in the classification of the early stages, but all parts are potentially important, including internal organs, although a sufficient material has seldom been available for studying the latter in a greater number

of related species and genera. An outstanding case for the importance of these organs is the study of sawfly larvae by Maxwell (101), whereas Beier (7) has shown that the central nervous system may be similar in unrelated, and dissimilar in closely allied beetle larvae. It has been said with some reservations that the end of the abdomen retains a more typical form than the mouthparts [Maulik (100)] and that the first or cephalic segments undergo a greater degree of specialisation than the following [Hinton (73)], but ontogenetically the head is older than the caudal end and should be expected to show more phyletic characters than the posterior end, which should tend to show more caenogenetic differentiations. Although characters of phylogenetic importance may be found on all parts of the body, a review of the literature shows that the structure of the head tends to remain more or less uniform in major groups, whereas modifications of the body segments and especially the hind end furnish as a rule a ready means for distinguishing genera, species, and even instars.

Significance for identification.—Of the three main tasks of taxonomy, the first, identification, has so far largely used the adult only, except often in mosquitoes, and the number of groups in which the majority, let alone the entirety, of the species of a country can be named in an early stage is still very small. As far as the larvae are concerned the existing literature suffices, however, to show that by and large their characters can be used in much the same way as those of the adults. Sixty species of larvae of the chafer genus *Phyllophaga* have been keyed by Böving (20), and the groups formed proved to be almost entirely congruent with those based on the male genitalia. Greater numbers of species have also been treated successfully in keys in the genera *Carabus* [Lapouge (92)], *Corymbites* (= *Ludius* of American authors) [Glen (64)], *Apion* [van Emden (49)], *Simulium* [Sommerman (130); Rubtzov (121, 122)], various genera of Culicidae, etc. True, there are not infrequent cases in which larvae of plainly distinct adults cannot (at present) be distinguished. However, by way of compensation not a few cases are known in which only one form of adult belongs to two distinct forms in the early stages, which are obviously two good, or at least biological, species, e.g., the beetles *Trachys griseofasciata* Saunders [egg, larva, pupa differing: Yano (146)] and *Meloë violaceus* Marsham [larva: Blair (13)]; various Indian Geometridae [larvae: Singh (129)]; the bombyliid *Hemipenthes morio* (Linnaeus) [pupa: Gäbler (59)]; some Blepharoceridae of the genus *Bibiocephala* [larva: Johannsen (82)]; the biting midge *Culicoides nubeculosus* (Meigen) [larva, pupa: Kettle & Lawson (84)]; mosquitoes of the genera *Tripteroides* [larva, pupa: Baisas & Ubaldo-Pagayon (4); Belkin (9)], *Orthopodomyia* [larva: Carpenter & LaCasse (29)], *Anopheles umbrosus* Theobald [?larva "*novumbrosus* Strickland": Swellengrebel & Rodenwaldt (132)]; and in Hemimetabola various species of Callaphididae [larva: Quednau (114)]. The significance of the distinctive pupal forms, the adults of which are not distinguishable, in Simuliidae remains problematic [Tonnoir (134); Freeman & De Meillon (57)]. Moreover, there are many groups of species in which the

adults are not easily separated, or identifiable only in one sex, whereas certain early stages are quite distinct. This is the case for instance in the tortoise beetles, *Physonota unipunctata* (Say) and *helianthi* (Randall) [larva, pupa: Sanderson (124)]; the chafers, *Chlorochiton suturalis* (Fabricius) and *longicornis* Arrow, [larva: Hoy & Given (77)]; certain Lepidoptera [pupa: Risbec (116); larva: Gardner (61)]; *Croesus* [larva: Benson (10)]; *Stilbula* [larva: Clausen (33)]; *Hermione* [larva: Vaillant (138)]; *Culicoides obsoletus* (Meigen) and *scoticus* Downes & Kettle, [larvae: Downes & Kettle (41)]; *Chironomus thummi* Kieffer and *dorsalis* Meigen [chromosomes of larva: Acton (1)], *Orthocladius rubicundus* (Meigen) and *abiskoënsis* (Edwards) and other Chironomidae [pupa: Thienemann & Krüger (133); Krüger (90)]; *Uranotaenia*, *Aëdes* [larva: Mattingly & Brown (99); pupa: Penn (109], *Ficalbia mimomyiaeformis* (Newstead) and *plumosa* (Theobald) [larva: Hopkins (76)] and other mosquitoes; various Ephemeroptera [larva: Burks (26)]; the coccids of the genus *Kermes* [larva: Balachowsky (5)]; and the Aleurodidae generally [resting larva: Trehan (135)]. Further examples of these and the following points will be found in a previous discussion [van Emden (47)]. On an infraspecific level some corresponding or congruent subspecies of adult and larva have become known, e.g., *Simulium tuberosum polare* Rubtzov [also pupa: Rubtzov (122)], *Philorus tianshanicus nivium* Brodsky [also pupa: Brodsky (24)], *Gomphus schneideri helladicus* Buchholz [Buchholz (25)], races of *Erebia tyndarus* Esper [Lorković (97)], *Aëdes aurantiacus* var. *nigrescens* Edwards [Edwards & Payne (45)], etc., but there are also cases in which two larval subspecies belong to one adult form, or in which larval subspecies are more conspicuously distinct than those of the adult. The three European larval races of *Cimbex quadrimaculata* Müll. [Benson (10)], two North American larval races of *Corymbites rotundicollis* (Say) [Glen (64)], the European races of *Porthetria dispar* (Linnaeus) [Goldschmidt (65)], two Mediterranean larval races of *Aëdes pulchritarsis* Rondani [Mattingly (98)], two American ootheca races of *Stagmomantis limbata* (Hahn) [Breland & Dobson (23)], and other cases illustrate this point, for which the plentiful literature on the *Culex pipiens* complex [e.g., Knight (85)] and the *Anopheles maculipennis* complex in which the eggs supply the decisive characters [Weyer (143)] is also relevant. Environmental forms, fluctuating variations in coloration and structure etc. have often been studied and usually been found to be uncorrelated to recognisable adult forms, but dark forms of green or grey phytophagous insects produced by crowding, which often show an orange or reddish-brown pattern, become in hemimetabolous insects like locusts a corresponding distinctive gregarious adult "phase" [Uvarov (137)], whereas in Lepidoptera the adult is not changed [Faure (54); Sevastopoulo (127); Long (96)]. Possibly, some of the "red mutants" of sawfly larvae mentioned by Benson (10) are also forms produced by crowding. Lastly, sexual dimorphism is of course widely existent in pupae but reflects practically [an exception was described by Zavřel (148)] always primary or secondary adult sexual characters. Even in larvae most of the sexual dimorphism consists in the gonads, or Herold's

and Ishiwata's organs, being visible through the integument of Lepidoptera and chafer larvae [Yagi & Kawada (145); Hurpin (78)], and the exterior genitalia affecting the shape of the posterior end in Hemimetabola. Greater size of one sex will imply larger larvae and pupae ("Tribolite" larvae, *Selasia*, etc.). However, striking genuine sexual dimorphisms of eggs and larvae have been described in Eulophidae by Flanders (55) and a different larval coloration of the sexes in *Anopheles barbirostris* Wulp by Rodenwaldt (117).

Significance for classification.—The importance of characters of immature insects for classification has been early and widely recognised, so that only a small selection of the many examples can be mentioned. The major classification of Odonata and Neuroptera has for a century been based almost equally on larvae and adults. Similarly, larval characters have early been used to support the primary division of the Coleoptera, Hymenoptera, and Diptera. In Aphididae important taxonomic progress has been made by the introduction of larval characters [Börner (18); Sampson (123); Quednau (114)], and in Coccidae the larvae supply characters for classification in many Diaspididae, especially the Aspidiotini [Borkhsenius (14)]. Ross (120) and Hickin (70) both dissolved the caddisfly family Sericostomatidae on the basis of larval characters, thus achieving a more natural arrangement of the families. Numerous valuable papers by Gerasimov on the classification of larval and pupal Lepidoptera suggest for instance certain changes in the scope of the Psychidae (63), from which among other groups the Acrolophinae must be excluded. Again on larval characters, Hinton (75) has since included these in the Tineidae. In Nymphaloidea the larvae appear to offer the most dependable basic characters for primary subdivision [Clark (30)]. The larvae of the Ostomatidae reveal that these are more closely related to cleroid beetles than to Clavicornia [Böving & Craighead (21)], and those of the Balginae belong so clearly to the Elateridae, that the subfamily must be transferred from the Melasidae, a change which adult characters confirm [van Emden (48)]. The position of the Niponiidae was doubtful until Gardner (60) discovered the larva and proved that it belonged to the Histeridae. It has also been possible by means of larval characters to elucidate the position of some isolated groups of Rhynchophora like *Urodon* (= *Bruchela*), Proterrhinidae, Belinae, and Rhinomacerinae [Anderson (2, 3); van Emden (49)]. Among Hymenoptera the Evaniidae cannot be separated in the larval stage from the Aculeata, and this agrees with the fact that it had been suggested before on the basis of the adults that they could not remain in Ichneumonoidea [Short (128)]. In Vespidae the larvae show that the Eumeninae and Vespinae on the one hand and the Polybiinae and Polistinae on the other are more closely related [Reid (115)]. *Mycetobia* had been classified with the fungus gnats until Edwards (44), after a study of the adult stimulated by a personal communication from Keilin on the characters of the larva, recognised that it must be placed in the Anisopodidae. Similarly, the relationship of the larvae enabled Dupuis (43) to state that *Aulacephala* is related to the Ormiini rather than Phasiinae.

The eggs have been used especially in Heteroptera for supporting divi-

sions or amalgamations of groups. The entirely different eggs confirm the view of those who separate the Urostylidae as a family of their own from the Pentatomidae [Miller (103)] and who consider Piesmidae and Tingidae or Nabidae and Reduvioidea [Leston (94)] as unrelated. The egg structure and the type of egg-burster also help to suggest the Pentatomomorpha and Cimicomorpha as the major divisions of the "land bugs" (Geocorisae) [Leston, Pendergrast & Southwood (95)]. A classification of the Coccidae into subfamilies can be based on the eggs (Putzhkova (113)]. In Acrididae, the Acrydiinae, Conocephalinae, and Decticinae seem to be well character- ized in the egg-stage [Tuck & Smith (136)], and from Jancke's (81) figures it appears that the classification of the sucking lice is well reflected in the structure of their eggs. Lepidopterous eggs are relatively well known but hardly yet truly classified [Döring (40)]. In Diptera the eggs of the Culicidae can be separated into groups corresponding to those based on the adults [Carpenter & LaCasse (29)], and in Coleoptera groups formed by the egg- cases of Hydrophilidae correspond as a whole with adult subfamilies etc. [Böving & Henriksen (22)]. Pupal classification vindicates the views of those who regard *Nomia* related to the Halictini and not the Andrenini [Michener (102)]. In Noctuidae subfamily characters seem to be more sharply defined in the pupae than the larvae [Gardner (62)], and in Trichoptera the struc- ture of the pupae confirms partly the soundness of taxonomic conclusions from the other stages [Ross (120)], not to mention the Diptera, the major classification of which is partly based on the difference between pupa and puparium. Pupal classification is especially advanced in Culicidae, Ceratopo- gonidae, and Chironomidae, and in the latter nomenclature is in many cases "rightly" [Thienemann & Krüger (133)] or "wrongly" [Hennig (68)] more elaborate than adult grouping.

In some cases outstanding characters of the early stages were the main reason for creating separate families, e.g., the stonefly family Peltoperlidae [Claassen (32)] or subfamilies, e.g., the blackfly subfamily Gymnopaidinae [Rubtzov (122)] or for erecting new genera. An interesting isolated eggtype confirmed the justification of segregating a stonefly as a new genus [Frison (58)].

Significance for phylogenetic studies.—Phylogenetic research is largely based on the same facts as classification, and to a large extent a natural classification is a summary of phylogeny. Many of the examples given above show, therefore, the significance of characters of immature insects for phylo- genetic considerations of a kind of which probably both the advocates [Yuasa (147)] and adversaries [Parker (106)] of this branch will approve. Morphologically the larva of *Micropteryx* is more closely related to the Mecoptera (=Panorpata) than to the moths, in which the adult has been placed, and in fact less closely related to these than are the caddisflies [Hinton (71)], so that *Micropteryx* must have separated from the panorpoid stem before Trichoptera and Lepidoptera became differentiated. It is gen- erally accepted that the Holometabola have evolved from Apterygota by

way of some hemimetabolous group, a conclusion which originated entirely from the characters of the immature stages as all the names "Hemimetabola" ("Heterometabola," "Exopterygota") and "Holometabola" ("Endopterygota") indicate. On the basis of their larvae and metamorphosis the fleas have been recognised as Holometabola closely related to primitive nematocerous Diptera almost generally since Lamarck (91) first separated them from the other "Aptera." The widely accepted view that the Coleoptera originated from primitive Neuroptera Megaloptera is mainly based on the structural similarities between the larvae of the latter and those of the Coleoptera Adephaga [Böving & Craighead (21); Crowson (37)], though it is supported also by characters of the adult. The close relationship of the paedogenetic Micromalthidae with the Cupedidae has first been discovered by larval characters [Peyerimhoff (111)], which also confirmed the position of the Cupedidae in or very near the Adephaga, thus changing substantially the current views on the phylogeny of the beetles [Peyerimhoff (112); Crowson (37)]. Most authors concerned with the evolution of the many families of the Coleoptera Polyphaga have made much use of larval characters, the phylogenetic trends of which have been discussed especially by Böving & Craighead (21), Peyerimhoff (112), van Emden (50), and Crowson (37). Evolution within one family of that suborder is very instructive in Lucanidae where the stridulatory apparatus, raster, mandibles etc. of the larvae show beautifully the change from the homogeneous to the differentiated and from the simple to the complicated condition [van Emden (52)]. Somewhat similar to the case of *Micromalthus* is that of the Orussidae in the Hymenoptera. This family has long been classed with the sawflies etc. in the Symphyta, but the larva was found to be parasitic and related to other larvae of the Apocrita [Rohwer & Cushman (118)]. A study of the adult soon afterwards led Börner (16) independently to include the family in the Apocrita. The characteristic evolution from a well-differentiated condition in the Nematocera "eucephala" to a secondary simplicity by reduction in the Cyclorrhapha is as pronounced in the larvae as in the adults of Diptera. After having worked out keys to some 130 genera of larval Cecidomyiidae, Möhn (105) discussed the phylogeny and showed that gall-forming midges occur only in the most highly evolved of the four subfamilies. In hemimetabolous orders, a study of the tracheal system of the larvae of Ephemeroptera and Odonata enabled Calvert (27) to trace the rudiments of the spiracles and the tracheae leading to them and thus to prove that the present closed respiratory system arose from an open one. The ancestry of the aquatic Hemimetabola must, therefore, have been air-breathing. Watson (140) found that a grouping of the dragonflies based solely on the larval mandibles exactly fitted Tillyard's phylogeny of the order.

Eggs and pupae also yield material for phylogenetic conclusions. The simple eggs of the Muscinae and Anthomyiinae [Collin (34)] are undoubtedly more primitive than the evenly flanged ones of the Phaoniini and especially those with hornlike flanges at the front end. In the caddisflies the females

of the primitive families enter the water to deposit strings of eggs held together by a thin "cement," whereas in more highly evolved families an egg-mass surrounded by a gelatinous water-absorbing substance is formed at the tip of the abdomen and then deposited [Ross (120)]. The phylogenetic importance of pupal morphology is exemplified by the Cerambycidae, in which all the more primitive groups have cerci [Duffy (42)], a trend also found in Coleoptera in general [Paulian (107)]. In coleopterous pupae evolution is equally conspicuous in a reduction of the number of spiracles and changes in the hair cover [Paulian (107)]. The relatively primitive phylogenetic position of the Attagenini in the Dermestidae is emphasized by their pupae, as these have preserved the cerci which the larva has lost [Hinton (72)].

Both in theory and in practice the characters of immature insects as a general rule prove to be of equal importance for the taxonomy of insects as the characters of the adults. By creating a wide possibility for classifying and identifying, this taxonomic significance attains importance in many other fields for which the identification of insect larvae is essential.

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CASTE DETERMINATION IN SOCIAL INSECTS[1]

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INTRODUCTION

This is a cursory survey of recent work. However, for full treatment there are a few references to earlier work which are essential (47, 48, 59, 66, 67, 87, 88, 89, 100, 101, 102). Only intrasexual polymorphism is considered, although internal anatomical and behavioural differences between outwardly similar adults are cognate and would have been included had space allowed. Determination may result from genetic or epigenetic influences; if the latter it may occur in the embryo (blastogenic) or in the larva (trophogenic), but physical factors such as moisture and temperature modify the organisms reaction to food. Food is, of course, a complex variable and one of the present needs is to analyse this and discover the relative importance of the total quantity, the rate of supply, the concentration or purity, the composition or the content of specific growth, and caste evocators. One addition to current terminology has been reluctantly made: the word "gyne" is used to denote a sexual female that is not socially a functional reproductive.

GENETIC DETERMINATION

In *Melipona* caste is probably determined genetically [Kerr (62, 63)]. Gyne and worker although distinct in form are similar in size and emerge from identical cells that are randomly distributed [Salt (90); Kerr (62)]. A random variation in food purity might exist since in the related *Trigona gribodoi* Magretti several shots of food are added to each cell by about five workers [Bassindale (1)], and it is likely that errors in the normal sequence of pollen, honey, and milk might be made, and the frequency of such errors might be species characteristic. However, the fact that gyne frequency may plausibly be 25 per cent in one species and 12.5 per cent in several others (values derived from the genetic theory that they are heterozygous for two or three alleles respectively) strongly favours genetic determination. But these proportions are only recorded in optimal conditions; otherwise more workers result, and one must suppose that either gynes are sub-viable or as Kerr prefers, reproduction is then by diploid parthenogenesis (giving no heterozygotes). For this there is no direct evidence yet. Since most gynes are killed the genetic process is wasteful, and this no doubt has been a factor on which natural selection has worked. Kerr believes that the genetic mechanism has supplanted a trophogenic one and evolved through a two-allele to a three-allele control, but it is difficult to accept the idea that any tropho-

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[1] The survey of the literature pertaining to this review was completed in December, 1955.

genic system would be forsaken for such a rigid and wasteful genetic one, and there seems no reason why the genetic process should not be primitive and have led to a trophic control such as is seen in *Trigona*. It is only necessary that when food is abundant large cells should be made, serving at first only for storage but later for brood rearing as well.

In higher termites it is possible that an evolution towards genetic determination may have occurred, for it is now known that some species of Termitidae form their soldiers from one sex and their workers from another another [Noirot (77, 78)]. Although some species retain the potentiality to form both castes from both sexes, some have lost it, so that in these cases it would be sex, and hence gene, determined.

BLASTOGENIC DETERMINATION

In many cases it has been implied that the embryo is caste plastic, and in others it has been shown that this is so. *Apis mellifera* L. was perhaps the first, but recently *Polistes gallicus* L. has been added to the list, for at 25°C. eggs that normally give workers give gynes [Deleurance (27)]. *Bombus pratorum* L. has also been added, for if foundress queens are supplied with workers and food some of the first eggs may yield gynes [Free (33)].

However, there are a few cases in ants in which egg size has been shown to influence the adult-size of workers [Goetsch (36, 37)], and there is one well established case of caste bias: the eggs of *Formica rufa rufopratensis minor* Gösswald (to be called *rufa*) give gynes if matured in winter and workers if in summer [Bier (6, 7, 8)]. Neither is determined, for the first if starved as larvae give workers, and the second if reared by the related *Formica rufa pratensis* (to be called *pratensis*) give gynes. Bier has shown that the winter eggs have much more ribonucleic acid; such blastogenic factors dominate caste in *rufa*, and worker and queen influences are subsidiary. It represents an approach to the state predicted by Flanders (30, 31, 32), but the differences in yolk supply although attributable to temperature during oogenesis do not appear to be affected by egg-laying rate; if anything it is the smaller summer eggs that are laid more quickly. This applies to *Oecophylla longinoda* Latreille too, for small eggs are produced so quickly that their reduction division fails, and larger eggs form more slowly [Ledoux (65)]. Furthermore, as Wilson (102, 103) has pointed out the small eggs are certainly plastic and the large eggs (which may also be plastic) normally form workers, the converse of Flanders' prediction. It is curious that a theory so valuable in parasitic Hymenoptera should be finding so little support in social forms.

In the higher termites (Termitidae) internal structural differences between alate and apterous castes can be detected in the first instar, but until biometric or experimental analyses are available earlier differentiation must remain doubtful. The inhibitory influence of alates of *Tenuirostritermes tenuirostris* (Desneux) on their own type has been traced to the moment of hatching and may occur earlier still [Weesner (98)].

Trophogenic Determination

Very little is known except for *A. mellifera* of how food in its various guises influences caste. There is evidence, even in the simplest wasp society which produces only a few workers, that quantity is only a condition not a determinant, and in the highest most complex and homeostatic societies which produce only a few sexuals as required, refined psychosomatic or humoral controls may exist. Discontinuity between caste forms seems to be achieved either by a physiological assessment in the larvae which switches those in excess of one value (a reserve status, hormone ratio, or a degree of asymmetry) into one morphogenetic path and those below into another [reminiscent of the "giant" *Drosophila melanogaster*, see Gabritschevsky & Bridges (35)] or by a social assessment which causes workers to change behaviour abruptly when a certain trophic interstimulatory or humoral state is reached.

Bees and wasps.—Female variation although continuous is more marked in larger colonies of Polybiine wasps [Richards & Richards (89)]. As colonies grow they trend from low worker/larva ratios to higher ones as a result of some periodicity in brood production and the fact that adults live a longer time than they take to grow. Sexuals are formed at high ratios and swarms emitted. It is interesting that sharp periodicity could lead to quick changes in worker/larva ratio and a minimized production of intermediates. Whereas no other discontinuity mechanism appears to exist in these tropical species, in the European *P. gallicus* there is a physiological one which causes a (imperfect) separation of nondiapause females that are active and whose gonads may develop if the foundress queen is removed, from diapause ones that are quiescent, but eat little protein and much syrup and that copulate but have small resting gonads. Although similar externally, the former are workers and the latter gynes which hibernate and then start new colonies [Deleurance (26, 27)]. The caste difference starts in larvae and is likely to be a result of a change in food composition or supply rate rather than in the total quantity, and as Richards suggested for the Polybiinae, might arise from a shift in the attentions of the queen from cell making and egg laying to brood rearing attributable either to the increase in larvae or to an intrinsic age change. Low temperatures at night applied only to the adults can cause a reversion to worker-production in gyne forming colonies. Whether this causes a change in nurse behaviour (Deleurance could not detect an increase in sugar ration to larvae) or a change in a glandular secretion is unknown, but a progressive change in the structure of the labial gland has been demonstrated [Deleurance (28)]. Caste discontinuity caused by a behavioural switch is conspicuous in Vespine wasps which change abruptly from small to large cells and often begin a new comb, but nothing is known of the cause. The resultant sharp drop in brood density may account adequately for the caste size difference.

In *Bombus* caste is again largely a question of size and diapause. [It has

already been stated that Free (33) has shown that in *B. pratorum* differences must start in larvae, and the gynes arise when nurses and food are plentiful. There are a number of peculiarities still unexplained, however; thus the worker/brood ratio in *Bombus argrorum* Fabricius rises through the season, though the average size of females does not, but rises sharply when gynes appear [Cumber (25)]. Moreover Brian (9) was unable to find significant correlation between worker size and the worker/larva ratio existing during larval life, yet in batches of eggs the gynes form centrally where better service would be expected. Not only is gyne production sudden, it is as in *Vespula* irreversible, and can be induced by taking the queen away. This also starts workers laying, and it is possible that this combined with the egg-eating suggested by Cumber [and recorded in some cases, see Free (33)] may be important; but in view of Free's results, it may equally well be irrelevant.

Caste is usually thought to be determined by food bulk in *Trigona* and *Lestrimelitta* (Meliponidae) which make special large cells containing more but similar food and from which gynes emerge [Kerr (62, 63)]; but, since the larvae feed serially on milk, honey, and pollen it may be that the greater quantity of milk when very young plays a vital part in gyne generation. ("Milk" is a useful word for a variety of nutritive glandular secretions given to juveniles).

In *A. mellifera* with caste differences and social control more highly developed than in any other type of bee, trophogenic determination has long been known to occur [see review by Ribbands (87)]. Larvae are plastic for a few days after eclosion, but those in normal cells then lose gyne-potentiality although they are not worker-determined, for if transferred to "queen"-cells they may give intercastes [Haydak (58)]. The behaviour discontinuity of workers does no more than create normal nutritional variants during larval life; it is during metamorphosis that distinctive forms are built up (with, for example, considerable degeneration of the larval ovary in workers) [Zander (104); Zander & Becker (105); Oertel (79)]. This reorganization is controlled by hormones; thus the distal segments of the hind leg buds of a gyne if transplanted into a worker abdomen or vice versa show partial host adaptation [Lukoschus (71)]. Pollen basket setae, and wax and scent glands, which are larger in the worker, are controlled by a secretion from the head. Although the corpora alata on account of their greater relative size in workers seem likely to be involved, proof is lacking. Prothoracic glands transferred from queen prepupae to worker ones prevent the normal development of the pharyngeal glands.

Bee-milk is produced mainly or entirely by the pharyngeal glands and given in abundance to gyne-forming larvae; it contains a concentrated lipo-protein and is rich in essential vitamins of the B group [see review by Johansson (60) 1955]. Larvae in normal cells are rationed after three days and may have their milk diluted with honey and variable amounts of pollen from the crops of nurses, and they are given no store after sealing. In spite

of these striking quantitative differences in food supply there is evidence that caste is controlled qualitatively for Weaver (97) has found that larvae reared in the laboratory from fresh bee milk gave gynes whereas those reared on the same that had been stored at 5°C. for a year gave large workers, though both were allowed surplus. Presumably some essential evocator decomposed during storage.

The more remote cause of gyne formation, the building of special cells, has also been studied experimentally. It may happen in the presence of a queen or when she is removed or dies. In the former case the causes are unknown, but two general theories exist [Ribbands (87)]: (a) either because food is surplus and cannot be disposed of (as when young bees are numerous and laying space is short) or (b) because of over-density as a result of not merely adults but of juveniles. Ribbands concludes that there is more support for the latter but that the conception of density requires analysis. Swarming is a normal method of reproduction and would surely be expected to occur without the development of abnormal conditions. For most of the time queens inhibit gyne cell formation; although a high degree of contact with the workers is needed, the inhibition can act through a gauze screen and when parts of the body only are present [Müssbichler (75); Butler (19 to 22)]. Information concerning the queen's presence can be transmitted from worker to worker but not through gauze; it must evidently involve a fairly complex sign [see Pain (82)]. Butler favours a sociohormone theory and maintains that a gyne-cell inhibitor is identical with the well substantiated worker-ovary inhibitor [Pain (80, 81, 82); Groot & Voogd (56); Voogd (96)]. The latter is chemically stable, persistent, and originates in the head, in all of which features it contrasts with Butler's "queen-substance"; surely there are either two hormones, or more likely, there is one hormone and one signal.

Ants.—The approach started by Ezhikov (29) and Wesson (99) has been continued by Brian (11 to 16). The latter, using *Myrmica rubra* L., was able by reducing mortality and by the culture of single larvae to show that they are sometimes plastic late into the third (final) instar, for their fate can be controlled through the food and nurse supply. This important instar can be conveniently divided into a sequence of stages whose procession is size independent; the brain moves slowly from the head into the thorax, and then the legs split transversely into first two then three segments. The rate at which this happens is remarkably invariable at constant temperature in gyne-forming females and was taken as standard. In this way it was found that larvae are plastic up to the stage of brain half-transit; then follows a phase when worker determination can occur (to brain 0.9 transit but mostly at brain 0.6 and brain 0.8 transit); then a brief phase of gyne potentiality but not determination (up to and including two-segmented legs, when starvation gives intercastes); and finally there is a phase of gyne determination. Large size relative to maturity, high specific growth rate, and large wing buds (relative to leg buds) are associated with a capacity to form

gynes. Worker determination is usually accompanied by an increase in the development rate, which passes off after a few days (unless metamorphosis supervenes immediately) and causes smaller adult size. There is also an inhibition of wing and ovary buds leading to adult aptery and subfecundity. In females, as compared with males, the gonad and wing develop late and are independent of the legs, for when large after-winter larvae are deprived of their gyne potentiality by incubating them without protein their leg buds grow, but their wings (and presumably ovaries) remain unchanged, whereas in the male both grow equally.

An interpretation in terms of an adult system parasitic on a larval system has been suggested [Brian (15)]. As long as the latter is well fed it is able to suppress premature and partial metamorphosis because of the growth and development of the former. This idea may be modified by subdividing the adult system into a dorsal set of rudiments (wing buds, gonads, and perhaps ocelli) and a ventral set (leg buds, mouth part buds, and central nervous system) which compete for metabolites perhaps through the intermediation of the endocrine system. When small compared with the larva, both sets are supposed to grow freely, but later metabolite supply is likely to become insufficient, and the dorsal set are then likely to be at a disadvantage on account of their later development (in females only). If metabolite limitation can be postponed, by lavish feeding under ideal conditions, until the ventral set has caught up with the dorsal set, queen determination can occur; otherwise the ventral set are supposed to dominate the supply and prevent all growth of the dorsal set (more effectively, the smaller they are) so that workers are formed. At a higher level it has been shown that large (gyne-potential) larvae can attract and even monopolise worker attention in the presence of smaller larvae [Brian (16)], so that as in so many biological systems size is of overriding importance in growth potentiality. Thus, gyne morphogenesis is conceived as unstable because of disunity of the adult system; worker determination is a transition into a stable state achieved by elimination of one of the parts. The development acceleration which occurs at worker determination and which causes the smaller worker size, is probably a later superimposed acquisition, because primitively female ants appear to have been dimorphic but of equal size.

Most gyne-forming larvae pass through diapause in the summer in a stage which corresponds with that in which worker determination occurs. Prior to winter these are the only two alternatives, and even in autumn after diapause is past, such larvae if artificially incubated with spring type workers give workers (after an exceptionally rapid development with very little growth at all). The low temperature of the winter, however, confers on them an ability for sustained growth and gyne formation, and this process has been called vernalisation to distinguish it from diapause development and to draw attention to a similar process in plants. Diapause is thus a stage which holds large larvae over until the next season; it is not essential in itself for there is evidence that some small larvae that do not reach that

state through neglect and coolness, may form gynes in the spring. Males show no diapause and no vernalisation.

Some progress has been made towards understanding the cause of diapause in *M. rubra*. It arises not from a climatic but from a social influence, acting probably throughout larval life and being manifested in the third instar at a stage, already mentioned, before which larvae though biased are still plastic. The seasonal brood cycle from nondiapause worker-forming to diapause and then to prediapause larvae, is paralleled by a similar physiological cycle in the worker population (and probably also in the workers as individuals) from gonad activity to gonad inactivity and fat body growth [Brian (10)]. It has been shown that spring type workers cause nondiapause growth and worker formation in plastic third instar autumn larvae whereas autumn type ones can cause diapause and that large diapause larvae are produced by the spring type workers after their nondiapause ones, when they are presumably really summer types, but only at moderate temperatures [Brian (14)]. The worker change besides being one to high and then back to low egg production appears to involve a change in feeding habits from proportionally more to less sugar. As would be expected this effect is reflected in the larval gut, [J. Weir (unpublished data)], since larvae are often, especially when small, fed on regurgitated food. This change in worker behaviour can be reversed by periods at low temperature, as normally occur in winter (compare the situation in *P. gallicus*).

In *rufa* spring eggs are gyne potential but give rise to workers when food or nurses are short [Gösswald & Bier (42 to 45)]. Worker-determination takes place in an early instar within three days at 27° C. for after this transfer to large groups has no effect. The reverse: transferring larvae from large to small groups after three days, produces no adults. It doesn't follow that they are gyne determined for they may be as in *M. rubra* (when intercastes will appear in a similar situation) only gyne potential. Spring eggs of *rufa* produced artificially throughout the year, if cultured by samples of a natural *pratensis* population, give gynes in winter and spring and for a short time in August, but at other times workers. Evidently, worker action varies seasonally, and the authors suggest that newly emerged workers cause the August resuscitation; the counterpart of this in *M. rubra* may be the induction of diapause. A further factor influencing caste in *pratensis* is the queen in whose presence gynes are not produced; in *rufa* which is pleometrotic queen influences are less, and, as earlier stated the egg seasonal variation dominates caste bias in this species. It is quite clear that sexual production comes under many influences of a social as well as of an ecological nature.

In the subtropical *Monomorium pharaonis* L. studied by Peacock and his colleagues, the straw colour of the gut content of larvae persists longer in gyne-destined than in worker-destined ones [Peacock & Baxter (83); Hall & Smith (57)]. The darkening, which eventually occurs, is associated with an increase in the amount of degraded haemoglobin compounds which are presumed to arise from the liver given as food, and it may be that this change

develops through starvation, which causes metamorphosis: if early, to give workers, if late, to give gynes [Eastham & Sudd (personal communication)]. Alternatively, it may be a sign of determination which is delayed in larvae fed on prepared protein (a high egg mortality is recorded when sexuals are being formed) and which yield gynes. Yet chemical analysis of the gut contents reveals no qualitative differences between sexual and worker larvae; amino acids and sugars appear much the same in both (but gamma-carotene shows signs of decreasing with age more in sexual than in worker larvae). Gynes and males arise simultaneously and can be produced at will by removing queens from flourishing colonies [Peacock *et al.* (84)] which normally would give only workers. Evidence suggests that male eggs are usually mixed in with female ones but are normally destroyed; it is curious that they are able to survive better when egg destruction in general is high.

The tropical army ants *Eciton hamatum* and *Eciton burchelli* produce about six apterous gynes along with thousands of males and no workers in a special brood at the start of the dry season, and then their colonies divide [Schneirla & Brown (95)]. The female larvae are much more attractive to the workers than the male larvae and dominate the food supply, which as in *M. pharaonis* is no doubt influenced by the concurrent brood destruction. These gynes although much larger than the workers, grow to maturity in less time (*A. mellifera* gynes are the only other recorded instance of this), thus caste determination which is presumed to be trophogenic hinges on what causes so few female eggs to be laid. No doubt climatic actions are important, and Flanders (unpublished data) has suggested that dryness depresses the queen so that the spermathecal valve is not opened during oviposition; a few of the first eggs might, however, be fertilized by sperms surviving in the oviduct from previous occasions.

The tropical genus *Oecophylla* has been studied by Bhattacharya (5), who maintained that polymorphism "is simply dependent on the quantity of food" on rather tenuous evidence, and by Ledoux (64) who has by many different methods made it highly probable that caste is determined trophogenically. In particular, as already mentioned, parthenogenetically produced diploid eggs laid by workers all apparently alike could give either gynes or workers. Fertilized queen-laid eggs normally give workers but are not necessarily worker-determined for the queen herself may inhibit gyne generation in some way as in *Formica* and *Monomorium;* certainly, female broods containing few or no worker-fated larvae at all usually occur in separate nests in multidomus colonies which contain no queens, and these are produced continuously in large but sporadically in small populations. Males were also produced in queenless conditions but in separate nests away from the gynes and from normal size haploid worker-laid eggs. Differentiation between gyne and worker occurs a few days after hatching as in *Formica*, and thereafter the gyne-destined larvae are white and sac-like with a mobile head separated from the body by a fold. The whiteness appears to be attributable to a special food, and as Ledoux comments on the wastage of young worker-destined larvae when sexuals are being produced, one may surmise that this represents

yet another case of brood destruction being associated with sexual production. Ledoux was unable to confirm Bhattacharya's claim that gynes can be produced in the laboratory by feeding the secretions of aphids and flower buds, nor were vitamin B, auxin, scale insects, and other special food efficacious.

The two sizes of worker seem to come from eggs laid by queens and by workers, as they are frequently mixed. A queen without workers gives rise to minors at first, then majors, but if she is allowed worker assistance from the start, she gives majors as well as minors, evidence of trophic differentiation [as in *Pheidole* spp., see Goetsch (36); Gregg (55)]. Ledoux has pointed out that since the minors are largely nurses of the majors, and the majors foragers, shortage of minors will tend to be self-regulating (as in fact happened in Gregg's comparable experiments). The difference between these two worker castes can be traced back into the second larval instar to very near the stage after which gyne-fated larvae can be recognised easily. This indicates that both may depart from gyne morphogenesis independently (the majors later than the minors) so that the system is comparable with that of *Myrmica* in which, as already stated, there are two principle stages of departure, the later one giving larger workers than the earlier although the total resultant distribution is unimodal. Soldiers are not necessarily homologous with large workers, and they may be formed by an allometrical extension of a single worker-generating deviation from gyne-morphogenesis [see Wilson (102)]. In this event their formation would resemble that of the analogous caste of termites.

The parasitic Hymenoptera show some striking parallels with ants. There is the well known case of *Trichogramma semblidis* the male of which is wingless when bred from some hosts and fully winged from others [Salt (91, 92)]; more recently there is the case of *Gelis corruptor* (Grav) in which the amount of food decides whether micropterous or macropterous males will form [Salt (93)]. Determination takes place between the early larval stage and the pupa. The cycle of *Melittobia chalybii* in a single host is very like that of *Myrmica* in a season; the first larvae feeding on the fluids give brachypterous adults, the later ones feeding on the solid tissues give winged adults, and those moreover pass about two months of the larval stage in diapause [Schmieder (94)]. The two alternatives are determined by the quality of the food received.

Termites.—Polymorphism is richer in the Isoptera; juvenile stages are useful and their growth can be arrested or regressed, and both sexes are socialized often with differentiation of function.

Our knowledge of caste structure and potentiality has been greatly advanced in the past decade by Grassé and his colleagues in France. In the comparatively primitive *Kalotermes flavicollis* Fabricius the work, such as it is, is done largely by an advanced larva which is plastic; it can become either an alate via a series of nymphal stages, or a brachypterous (substitute) reproductive by a development of the gonad with one moult, or a soldier by a hypertrophy of the anterior region and a degeneration of the gonad with

two moults the first of which leads to a pupa-like stage called the pre-soldier. This type of ontogeny applies to most lower termites of the families Calotermitidae and Termopsidae [Grassé *et al.* (53); Noirot (76)]. Comparative sociology and morphology suggest that from this stage there evolved one in which an alate line with full sexuality separated from an apterous line with restricted sexuality, the dichotomy starting earlier and earlier in ontogeny and becoming more irrevocable. Thus in the Rhinotermitidae it starts after two larval moults and in the more advanced Termitidae after only one moult (or, as previously mentioned earlier still). Whereas in the former family (and some of the latter) a worker can become a substitute, sexual it takes two moults instead of one. In the higher Termitidae this power appears to have been lost altogether [Buchli (17); Noirot (76, 77, 78)]. Even the alate path is not determined in Rhinotermitidae, for nymphs of *Reticulitermes lucifugus* Rossi if badly fed regress and become workers [Buchli (18)]. Concurrently with these changes the apterous line of development becomes more specialised: gonad development is suppressed and helpless white larvae change more and more suddenly into active pigmented workers, and once changed they become more fixed, that is, they moult less and are less liable to change into soldiers. They retain their thoracic glands but curiously enough it is reported for one species that so do the soldiers [Pflugfelder (85); Noirot (78)]. [Thus the evolution of the apterous line appears to resemble that of the whole insect group; for the primitive Thysanura and Collembola grow gradually into adults which moult throughout life and retain thoracic glands, see Gabe (34)]. A very interesting point is that this worker fixation is partly socially imposed, for if removed from the colony they will pass into instars that they do not normally attain [Noirot (78)]. Associated with these changes and probably causally connected, is the evolution of a differential sex bias towards soldier formation; under natural conditions sometimes one and sometimes the other sex form soldiers (it usually being a family characteristic). In most cases, however, the normally nonresponsive sex retain the potentiality and in pure culture will give soldiers [Noirot (78)]. This may be partly attributable, of course, to their greater degree of moulting when away from the colony. Thus, whereas in the lower termites the worker is ontogenetically at the root of all castes; in the higher ones, it may only become a soldier and that rarely or never, depending on its sex.

The production of alates for dissemination as in ants is very much under the control of climatic factors. Even in equatorial rain forest there is a seasonal cycle, and the period of dissemination may last only a week, being in general shorter the more advanced the society [Grassé (47); Noirot (77)]. A possible explanation of this may lie in the inhibitory effect of alates (other than the king and queen) on the development of their own kind for Weesner (98) has evidence that in *Tenuirostritermes tenuirostris* they block the alate path of development at its bifurcation from the apterous one; this was also suggested for *Eutermes* by Bathellier (2). It is interesting to recall that alates also inhibit themselves or are inhibited by other components of the society [Grassé (46)[. In such an event the release of one lot is a signal for the generation of the next lot. They would then be likely to be very much of an

age, and in *T. tenuirostris* they are all in the fifth instar in winter. It is generally agreed that colonies must reach a certain size before they yield alates at all, but as might be expected the minimum number even for *K. flavicollis* is said to vary from 20 to 100 according to different authors [Becker (3); Grassé (47); Lüscher (74)].

It is well known that the reigning pair inhibit the formation from juveniles of brachypterous substitutes (alate in some Termitidae), but beyond the fact that contact is necessary for this, there is little agreement. Attempts to show the existence of a sociohormone [first apparently suggested by Bethe (4) and Pickens (86)] by administering extracts of reproductives have failed [see Light (66, 67, 68); Keene & Light (61)]. The alternative, an inter-individual sensory stimulation acting through the central nervous system on the humeral system, is difficult to prove [Grassé & Noirot (50, 51, 52); Grassé (47, 49); Noirot (76, 77, 78)]. Such are known to occur in non-social insects, and in primitive social insects (*Polistes, Bombus*) there is now good evidence that general activity differences, probably genetic in origin, cause social hierarchies which influence the size of internal organs and the behaviour of individuals, but in *A. mellifera* there is little doubt that the same effect is achieved by a sociohormone.

In *Zootermopsis angusticollis* Hagen the longer the primary pair are removed the greater the proportion of larvae that become supplementaries [Light & Weesner (69)]. In *K. flavicollis* 24 hr. absence is enough to start regeneration, and each individual is especially sensitive to induction immediately after a moult [Lüscher (72, 73, 74)]. But response is probably modified by other factors; in *Termes hospes* Sjöstedt not all in the sensitive post moult period respond [Noirot (78)], nor do they in *Z. angusticollis* [Light & Weesner (69)], and in *R. lucifugus* although sensitivity lasts from the first third of each instar, it is apparently reduced in old individuals and those with low fat body reserves [Buchli 17, 18)]. It is a curious fact that the sex-specificity of inhibition appears to be imperfect, for with *Zootermopsis* the removal of one sexual leads to replacements from both sexes but with a definite bias in favour of the sex removed [Castle (23, 24); Light (67); Light & Weesner (69)]. In *K. flavicollis* the same appears to be true [Grassi & Sandias (54)], a fact which may account in part for the apparently opposite statements of Grassé & Noirot (50, 51) and of Lüscher (73, 74).

A colony divided by an impermeable partition or by double gauze develops substitutes in the alateless half, and all but a pair are destroyed, but if the gauze is single so that contacts occur through it, although substitutes are formed they are all destroyed [Lüscher (72, 73, 74)]. One may infer that the inhibition cannot pass but that information about the existence of alates can pass. Lüscher supposed that the information was transmitted by a simple sensory process and that the inhibition was a result of a sociohormone whose passage across the gauze failed on account of some difficulty in transmission (licking may be awkward for example). It might also be attributable to a more complex sensory process, for example, an orientated action or signal which is capable of causing the necessary change in behaviour or metabolism. The situation is in fact reminiscent of that between sexes of flies, the male

can readily perceive a female through gauze but might fail entirely to induce copulatory behavior because of the difficulty of transmitting his courtship.

It appears that the creation of these substitutes is only conditioned not determined by food, for a nymph of *Z. angusticollis* if isolated will develop gonads although it fails to mature [Castle (23, 24)] and pairs of nymphs of *K. flavicollis* form substitutes [Grassé & Noirot (50, 51)]. In the latter case although saliva replaces normal food, it only does so gradually after the first moult, too late to determine the change. In *R. lucifugus* social and mechanical disturbances are able to induce sexualization of larvae; in this species the reigning alates appear to have no inhibitory influence and are soon supplanted under normal conditions by substitutes which are more attractive to workers [Buchli (17, 18)].

Very little progress has been made in analysing the process whereby soldiers inhibit the generation of their own caste. In addition to the now classic exmples of this, in relatively primitive families, it is recorded in *Reticulitermes hesperus* Banks of the Rhinotermitidae [Light & Weesner (70)] and in a number of genera of Termitidae [Noirot (78)]. Goetsch (38 to 41) has claimed to produce soldiers in *Anoplotermes*, a genus normally without such, by feeding protein rich diets, as with the ant, *Pheidole*, and watersoluble vitamin extracts (called vitamin T but similar to one of the B series in properties) as well as antibiotics, but it is widely held that a mistake in the identity of the species was involved [Light (66, 67); Grassé (47)]. Nevertheless, the known effects of these substances, especially vitamin B_{12} and antibiotics, on the growth of vertebrates suggests that this work might profitably be pursued further.

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DYNAMICS OF INSECT POPULATIONS[1]

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So short a review of such a complex subject is necessarily sketchy and selective. It is confined almost exclusively to work of the last 10 years and by necessity omits many valuable contributions within this period. I have chosen sometimes arbitrarily from among those which seemed most significant for the main themes of density-relationships and natural control.

In using the term "population" I shall mean all the members of one species in a particular "environment," the limits of which are a matter of convenience, but wide enough for the population and its associated species to be considered as an ecological unit. "Population density" will mean either local density, or mean density (or abundance) over a space in which the population may be scattered in groups. "Competition" is defined on p. 126, "density-dependence" on p. 131, "natural control" on p. 132.

THE POWER TO INCREASE

The power to increase in favourable conditions, inherent in all living things, is the mainspring of population dynamics. It has often been calculated from the data of laboratory experiments in which the schedules of development, reproduction, and survival have been determined. There has recently been an increased interest in methods of doing this, and Cole (1) has given an excellent comparative account of them. For most purposes the best available method is one based on life- and fecundity-tables and adapted for entomological use by Birch (2), Leslie & Park (3), and Howe (4). It assumes that the unhindered growth of a population which has settled down into a stable proportion of different age-groups is geometric, the numbers increasing by a constant factor λ per unit of time (e.g., per week); this factor is called the "finite rate of increase." The "intrinsic rate of natural increase," r, which is more often used, is defined by the relationships:

$$dN/dt = rN, \quad \text{or} \quad N_{t+1}/N_t = e^r = \lambda, \quad \text{or} \quad r = \log_e \lambda.$$

It has been renamed the "innate capacity for increase" by Andrewartha & Birch (5), who use the symbol r_m, reserving r to signify the actual rate of increase achieved by a population in nature; r_m is an index of increase of a species under a specified set of physical conditions, given adequate food supply and space. Another, less accurate, way of estimating the capacity for increase is to follow the growth of a culture in its earlier stages, before density-effects become prominent (1, 6). Any reliable measure of the possible rate of increase of a species has significance for ecology and economic entomology. However, an index such as the innate capacity for increase has severe limitations, which have been fully examined by Andrewartha &

[1] The survey of the literature pertaining to this review was completed in May, 1956.

Birch (5). Firstly, like other measures of increase, it applies only in the conditions in which it is determined; at other temperatures, or with restricted food, or with crowding effects or interference from other species, the rate of increase will be different. Secondly, it applies only to a population with a stable age composition, which is perhaps not often attained by natural populations.

If we enquire how differences in the schedules of development, reproduction, or survival influence the net rate of increase, we gain a much improved insight into their ecological significance. Birch (2), Howe (4), and Cole (1) have shown the relative influence of some of these differences upon the innate capacity for increase.

Differences in the power of increase may be expected to influence the relative abundance of different species under similar conditions or of the same species under different conditions. The efficiency of insect parasites as controlling agents depends greatly on their capacity for rapid increase [Clausen (7)]. The success of seasonal populations, which make a fresh start from low numbers at the beginning of each growing season, may depend a good deal on their ability to multiply quickly. Similar considerations apply in warehouses and granaries, where there is periodic removal of goods, cleaning up, and chemical treatment (8). Howe (9) compared the capacities for increase of nine species of ptinid beetles infesting stored products in Britain. The one with the highest rate (at 25°C.) was *Ptinus tectus* Boieldieu, which is by far the commonest. Birch (10) cultured *Rhizopertha dominica* Fabricius and the large and small "strains" (sibling species) of *Calandra oryzae* Linnaeus separately and together in species-pairs. Under each set of conditions, the species with the higher rate of increase in unmixed culture eliminated the other from the mixed cultures. At 29.1°C., the large strain of *C. oryzae* was dominant on maize, the small strain on wheat. On wheat, the small strain was dominant over *R. dominica* at 29.1°C., and vice versa at 32.3°C. As Birch remarked, between some other species the outcome of competition might be determined by interference, in which case it might not be well correlated with the rate of increase. In some circumstances, as we shall see for example in Nicholson's experiments with blowflies (11, 12), a high rate of increase may be disadvantageous, exposing the population to severer stresses and high mortality.

While the innate capacity for increase, and similar statistics, are figures of very limited application, they give an approximate measure of actual characteristics of the species concerned which enter fundamentally into its population dynamics, however greatly their expression may be modified in nature.

INFLUENCES ASSOCIATED WITH LOW AND HIGH DENSITY

Our present knowledge of the dynamics of populations in the field is based chiefly on populations at relatively high density. Species which remain at low density are very difficult to study, and the same applies to popula-

tions temporarily reduced to low numbers. When a population is sparse, intraspecific competition and shortage of requisites are less likely to occur. Natural enemies and disease may be less important than when the density is high, though not necessarily so; it depends on the recent history of the population and on the state of associated species. The chance of mating may be significantly reduced at low density. These matters have been discussed by Andrewartha & Birch (5) and briefly by Solomon (13).

The unfavourable influences associated with high density include crowding effects, increased cannibalism, shortage of food, impairment of the environment, and dispersal to less favourable places; increased liability to attack by natural enemies and disease will be dealt with later. High density may also bring certain advantages.

There is no space here to review the well-known work on density effects in laboratory cultures of insects such as *Tribolium*, *Drosophila*, *Callosobruchus*, and various grain pests. Accounts have been given by Allee *et al.* (14), Andrewartha & Birch (5), and (in part) by Solomon (8). The effects include reduced rates of development, survival, reproduction or net increase, and reduced size of individuals. They arise either as a direct result of crowding or from shortage of food or from "conditioning" of the medium.

Less is known about density effects in natural populations. In many species crowding or food shortage seems never to occur, and in some it is only a temporary phenomenon at the peak of a density fluctuation. Catastrophic droughts or defoliations may temporarily reduce the food of many species so that there is competition for what remains. A heavy infestation of fruit tree red spider mite, *Metatetranychus ulmi* (Koch), tends to destroy its food supply, with a consequent great reduction in the production of winter eggs [Kuenen (15)]. Insect parasites and predators may suffer both from the difficulty of finding their hosts or prey and from competition for them. Some species, whose food occurs in isolated units, suffer frequently from local overcrowding. The best-known example is that of the blowflies which feed on carcases, and whose larvae normally suffer a high mortality because far more eggs are laid than can develop to maturity on the food supply. As a result of the intensity of competition in these insects, the number which survives is far smaller than the food could support. This wasteful type of competition, in which much of the food is used by individuals which get less than enough fur survival, is called "scramble" by Nicholson (12) and is exemplified in his experiments with *Lucilia cuprina* Wiedemann. The type of competition in which an individual either survives or else uses none of the requisite, he calls "contest." This distinction could also be applied to the relationship between natural enemies in exploiting their hosts or prey; e.g., *Mormoniella* produces fewer offspring above a certain parasite/host ratio, because of superparasitism [De Bach & Smith (16)].

A dense population of insects, or even a rather sparse one in a situation from which heat cannot readily escape, may produce enough metabolic heat to warm the environment. The process is self-accelerating. Insects in

bulk grain sometimes cause local heating from which they either die or escape into cooler grain (17, 18). Blowfly maggots in a carcase may be so crowded and active that they generate enough heat to kill themselves (19).

SATURATION DENSITY LEVEL

Established laboratory cultures, in a constant amount of medium frequently renewed, tend to fluctuate rather than to maintain a steady high density. One can however consider the mean density or the mean of a series of peaks. The main points of interest are (a) to compare different saturation densities in parallel with other aspects of performance and (b) to find which density effects chiefly determine the saturation level in a given species, and here we must include food shortage.

Park's recent work (20, 21, 22) deals partly with the density reached in cultures of *Tribolium confusum* Duval and *Tribolium castaneum* Herbst. For some unknown reason, the mean adult density of *T. confusum*, at least, was significantly less in larger volumes of flour than in smaller volumes. When *T. castaneum* was freed of the common coccidian parasite, *Adelina tribolii* Bhatia, its population density was nearly trebled, whereas *T. confusum* did not respond in this way; sterile cultures of both species had a greater proportion of adults (20). Park (21, 22) compared the densities of the two species cultured separately, at various temperatures and humidities, with the outcome of the competition between them in mixed cultures. The results were complex and sometimes variable. Under some conditions, *T. confusum* had the higher density when single-species cultures were compared, and it was the surviving species in most of the mixed cultures. Under other conditions *T. castaneum* was superior in both respects. But at 24°C., 70 per cent relative humidity, while *T. castaneum* had substantially the higher density, it was *T. confusum* which survived in most of the mixed cultures; and at 34°C., 70 per cent, while there was no significant difference between the densities of the two species grown separately, *T. castaneum* was the survivor in all the mixed cultures. Hence the connections between the separate saturation densities and the competitive success of these two closely similar species are too indirect or complex to provide a reliable basis for predicting one from the other.

More straightforwardly, we may compare saturation density with other aspects of performance in unmixed cultures. In discussing his experiments on grain insects, Birch (23) posed the question, whether the peak density level is correlated with the innate capacity for increase. *Rhizopertha* and the two strains of *C. oryzae* were cultured on periodically replenished grain at two or three different temperatures at which the value of r_m for these species had been determined. *Rhizopertha* had the same peak density for two different values of r_m, but in both forms of *C. oryzae* there was a strong positive correlation between the peak density attained and the value of r_m. Birch constructed a hypothetical model to show one way in which such a result might arise. But since the rate of increase and the peak density are each the

resultant of a complex of processes, there may well be no reliable correlation between them in most cases.

Fluctuations in numbers imposed by weather are discussed elsewhere in this Volume (24).

One cause of downward fluctuations is that natural enemies often become more effective at high host density and finally cause a decline in numbers. Outbreaks of disease are often generated and spread more readily at high population density. At low host density, enemies and disease tend to become less effective, so that the host may be able to increase again. Mathematical models show a continuous cycle of increase and decrease, with the cycle of enemy numbers lagging out of phase with that of the host. A regular cycle of this sort may be a rare occurrence, but a single rise and decline, with the enemy population rising and falling a fraction of a cycle later, is sometimes observed in the field (25, 26, 27). Continuous and rather irregular fluctuations of a cowpea beetle cultured with various insect parasites have been demonstrated in the laboratory by Utida (28, 29, 30) who also showed that the amplitude of fluctuation was greater in a host with a higher rate of increase (31). In similar experiments with the moth *Ephestia cautella* (Walker), Takahashi (32) found that a parasite with higher reproductive powers and host-finding efficiency caused more violent fluctuations.

Intraspecific competition can give rise to fluctuations, as in Nicholson's experiments on *Lucilia cuprina* (12). When the supply of protein food for the adults was restricted, competition, with great wastage at high densities, led to an inverse relation between the numbers of flies in a cage and the numbers of eggs laid. But because of the time lag between the laying of the eggs and the emergence of the resulting adults, the flies alternately far overshot and undershot the equilibrium level: when there were many adults, competition for the food was so severe that few eggs were laid, so the adult population was not replenished and fell to a low level; this relieved competition, many eggs were laid, and the population of adults again rose too high. Similar oscillations were produced by restriction of the larval food. For comparison with the predator-prey type of fluctuation, it should be noticed that here also, in constant conditions, a steady population might be maintained, were it not for the time lag in the development of the full effect of each change in density. However, a steady density is not reached in cultures of insects in flour and similar media, at least not in the course of a few years. In most populations of this sort there is an obvious reason for fluctuation: the culture is set up with adults only, or with some other age distribution which is bound to undergo cyclic changes for a long time afterwards. In *Tribolium* [Park (20)] this matter is greatly complicated by the fact that the larvae and adults eat the eggs and pupae; and furthermore, the coccidian *Adelina* is commonly associated with *Tribolium* and influences its mortality and age-distribution. A valuable and long-sustained study of annual fluc-

tuations in the field has been made on pine defoliators in Germany [Sch-werdtfeger (33); (see also 34)]. On the theoretical side, Nicholson (12) considered possible types of reaction to limiting shortages and deduced a set of different patterns of fluctuation.

DISPERSAL

Dispersal, or movement from place to place, is discussed at length by Andrewartha & Birch (5) whose theme is that dispersal is a normal activity of most species at some stage in the life history and is often independent of population density. This proposition, with which few would disagree, is developed partly by reference to recent work on insects. In three of their examples, Davidson & Andrewartha (35, 36) on *Thrips*, Dobzhansky & Wright (37, 38) on *Drosophila*, and Kettle (39) on *Culicoides*, dispersal appears to have been independent of density. In the experiments of Ellis (40) on caged nymphs of the migratory locust, their "marching" activity depended on, among other factors, their density and their phase status, the latter depending on the density conditions in which they had been reared.

Various aspects of dispersal in relation to population density have been investigated by Itô (41, 42), Miyashita (43), Morisita (44), and Ootake (45). For example, Itô (42) placed two species of aphids on a single barley plant, one at the top, the other on the basal leaf. At first they remained separate, but on approaching saturation in their initial (and preferred) niches, they invaded the whole plant and lived side by side. When the joint population on the plant reached 30 to 60 per 10 sq. cm. of leaf, some migrated to surrounding plants, leaving an equilibrated population in which the ratio of the two species depended on the temperature. When another plant was close at hand, the aphids spread to their preferred sites on the second plant rather than to less favoured sites on the original plant. Itô concludes that dispersal is an important factor in maintaining population equilibrium and an alternative to eliminative competition. Dispersal of the fruit tree red spider mite is also influenced by population density [Marlé (46)]. as is that of *Ephestia* larvae in laboratory cultures [Takahashi (47)].

A population may spread into less favourable or only temporarily favour-able areas in response to some effect of high density. The spruce budworm does best on balsam fir trees with male flowers, but as defoliation progresses, many larvae disperse to nonflowering trees [Blais (48)]. Intolerance of crowd-ing drives grasshoppers into less favourable habitats [Pepper (49)].

The power of dispersal is a most important factor in the success or failure of insect parasites and predators as agents of control [Flanders (50); Clausen (7)].

INFLUENCE OF OTHER SPECIES

Interspecific competition.—Competition, i.e., rivalry between individuals or populations for requisites such as space or food has been little studied in the field, but many laboratory experiments have been performed on com-petition between different species under rather crowded conditions. In

Park's experiments with *Tribolium spp.* (20, 21, 22), as we have seen, the outcome of competition was influenced by the physical conditions and by the coccidian *Adelina.* In experiments with two species of *Drosophila,* Moore (51) found the outcome of competition was reversed by a change from 15° to 25°C. A change of 3.2°C. was sufficient to reverse the result of competition between *Rhizopertha* and *C. oryzae,* small strain [Birch (10)].

With some pairs of species, earlier work has shown that the outcome of competition depends on the relative densities at the start. This was not so in Park's experiments, where a change in the initial density ratio from 1:3 to 3:1 made little difference to the final result. The nature of the foodstuff must often be an important factor [Birch (6, 23)].

The above are some of the conditions which may determine the outcome of competition. For a direct analysis of the process we must examine how competing populations influence one another. Birch, Park & Frank (52) investigated the influence of crowding on the net fecundity rates (eggs laid minus eggs eaten) of *T. confusum* and *T. castaneum.* In separate culture the net fecundity of both species was reduced in the same proportion by a given degree of crowding but was always higher in *T. castaneum.* In mixed cultures the influence of *T. confusum* in reducing the net fecundity of *T. castaneum* was greater than that of *T. castaneum* upon *T. confusum* or of either species upon itself, all these lesser effects being about equal. In consequence, the average ratio of net fecundity of *T. confusum* to *T. castaneum* was changed from 1:1.28 in single-species culture to 1.33:1 in mixed cultures where the species being assessed was crowded with seven times as many of the other species. This effect of competition on net fecundity could not be accounted for by cannibalism, for neither species showed any preference for eggs of one or the other. The net fecundity of either species was reduced more by crowding with females then with males. This may have been a result of the much higher rate of egg-eating by the females, later demonstrated by Rich (53) in *T. confusum.* In view of the same fact, the authors' conclusion (52), that the effect of crowding by females was partly attributable to a direct effect of crowding on real fecundity, needs to be re-examined.

Another analytical approach is to enquire whether the outcome of competition is highly correlated with aspects of population performance. In *Rhizopertha* and the two strains of *C. oryzae,* as we have seen, Birch (10) found that competitive success was highly correlated with the rate of increase, whereas it was poorly correlated with the density reached in unmixed cultures in Park's experiments with *Tribolium spp.* (20, 21, 22).

In most experiments on competition between insects in grain or flour, acute shortage of food is avoided by periodic renewal of the medium. But in experiments on blowflies, a larval population has a single piece of meat, which is not renewed, and which is very inadequate for the needs of the denser populations. This is also the case in carcases. Waterhouse (19) showed that in Australia during the warmer months *Lucilia cuprina* lays numerous eggs on dead animals, but competition, particularly with other

species, and the lethal temperatures generated by the dense populations, prevent all but a few larvae from completing their development. Besides competing for food, *Chrysomyia rufifacies* Macquart eats or drives out the larvae of *L. cuprina* and other flies, and a high proportion of the survivors may be eaten by predatory beetles. *L. cuprina* breeds much more successfully on living sheep, where it meets far less competition and is not subjected to a concentration of predators when it drops to the ground. Ullyett (54) made an experimental study of competition between pairs of species of South African blowflies. In terms of biomass, the death-rate in *Lucilia sericata* Meigen on a limited quantity of meat was the same for any particular degree of crowding, whether by its own species alone or when half the population was *Chrysomyia chloropyga* Wiedemann. But when one species was a facultative predator upon the other, as well as competing for food, the second species suffered high mortality and a severe reduction in the size and fecundity of any survivors. The higher the initial density, the shorter and sharper was the competition and the smaller its effect on the mortality of the dominant species.

In nature many pairs of species compete for food in the sense that they share the same food supply, but so long as there is no shortage, so long as the numbers of the two species are kept down by other factors, competition for food will be unimportant and will not prevent the coexistence of the two. Only if two species are both limited by the supply of a common requisite, which they are both able to exploit in all the places where they occur, does it follow that one of them will be eliminated. These conditions must rarely apply to pairs of species in nature. Even in laboratory experiments, a small difference in habits, or a simple irregularity in the environment, may allow species to coexist, as Crombie (55, 56) showed in experiments with insects living in grain or flour. Crombie (55) maintained such a mixed culture, with *Rhizopertha* and *Oryzaephilus surinamensis* (Linnaeus) on broken wheat, replenished at intervals. The larvae of *Rhizopertha* live and feed inside the grain, those of *Oryzaephilus* outside, as do both the adults for the most part. Their coexistence is possible because they do not actively harm each other and because their food requirements are a little different. It is well known from Gause's experiments that two organisms may coexist if a refuge is provided for the one which tends to be eliminated. Crombie (56) applied the principle to cultures of *Tribolium confusum* and *O. surinamensis* in flour; the first species, being the more actively predatory, eliminated the second; but the addition of small glass tubes, into which the developing *Oryzaephilus* could crawl while *Tribolium* could not, allowed both to persist. Field populations of *L. sericata*, although greatly reduced by larval competition with the predatory *Chrysomyia albiceps* Wiedemann, are favoured by the habit of pupating deep in the soil, so largely avoiding the parasite *Mormoniella*, while *C. albiceps* does not escape in this way [Ullyett (54)]. Merrell (57) found that two species of *Drosophila* could coexist in cultures by virtue of a periodic change to fresh food; one species was favoured by stale food, the other by

fresh. When Utida (58) cultured two species of cowpea beetle (*Calloso-bruchus*) together, one was soon eliminated, but when their densities were depressed by a parasite, both persisted, at least for the six generations of the experiment. We also saw that a small difference in temperature or food material may determine which of two species will survive, and that the elimination of one of a species-pair may take years even under constant conditions favouring continuous activity. In view of the above points, it seems unlikely that "Gause's hypothesis" (that species of identical require-ments, identical ecology, or similar ecology cannot persist together) would apply to insects in nature [cf. Andrewartha & Birch (5)].

Parasites and hosts, predators and prey.—The interaction between natural enemies and their hosts or prey, which has been the subject of various mathematical theories, laboratory experiments, and a few field studies can only be touched on here. The host or prey is the food supply of the para-site or predator, so that if there is any shortage each tends to act as a density-dependent factor upon the other, though commonly with a time-lag between action and reaction [Varley (59); Solomon (13)]. But the influence of nat-ural enemies is not necessarily density-dependent: changes in weather etc., the presence of alternative hosts, and many other contingencies may prevent such a relationship, or a natural enemy may be unable to keep up with the increase of the host. The density-relationships between certain parasites and hosts have been investigated in laboratory experiments by DeBach & Smith (16), Burnett (60, 61), and Utida (62, 63).

Competition may occur between species of parasites; DeBach (64) made a field and laboratory study of two species of *Aphytis* one of which was dominant. But the variability of natural conditions and the common faculty of avoiding hosts already parasitised, and other circumstances, frequently allow a complex of parasitic species to continue sharing a common host (59, 65).

A parasite cannot reduce a host population to a low level unless it has sufficient ability to disperse and rapidly discover the hosts [Flanders (50); Clausen (7)], to be active at the same time as the host and in the same places [Thalenhorst (66)], and to develop and reproduce rapidly and con-tinuously while the host is active [Flanders (67); Clausen (7); Varley (68)]. The time required for an introduced parasite or predator to become effec-tive, and related matters, have been discussed by Clausen (7), Thompson (69), and Sellers (70).

In recent years the importance of ants in increasing the population level of many insects, chiefly Homoptera, by protecting them in various ways has attracted much attention (71 to 75).

Pathogens.—The role of disease in insect population dynamics has been discussed by Steinhaus (76). The following are some of his chief points. There is a firm impression among those experienced in this field that the in-cidence of disease is correlated with high population density. But few quantitative studies have been made, and it is known that disease may

break out at low density and that an epizootic which breaks out at high desnsity may then spread widely, regardless of density. Nevertheless, disease is essentially a density-dependent factor. But its controlling effect is often transitory:

Only in certain instances can microorganisms be established in a manner comparable with that accomplished with insect parasites and predators. Most applications of microorganisms are made in a manner analogous to the application of insecticides. Furthermore, natural outbreaks of disease are usually of a fulminating nature, and of comparatively short duration although the pathogen may be present in the population between epizootic periods.

Finally,

it would appear that disease usually has a higher threshold of operation than do insect parasites in a given population.

COMPLEX RELATIONSHIPS

Examples in other sections give glimpses of the complexity of population dynamics in nature. Even in laboratory experiments, like those of Park on *Tribolium*, the relationships may be very complex. In Nicholson's experiments on *L. cuprina* (11) involving one species with constant physical conditions and food supply, the microcosm was complex enough to exemplify the principle of compensation: that if one controlling agent relaxes, others become more effective, or if one operates more severely, others relax. In cages where the population was limited by restricting the supply of food for the adults, if a high mortality was artificially imposed on the adults at emergence, this relieved the competition for food and space and so led to the production of a greater number of eggs, with a consequent increase in the number of emergent adults. Similarly when the larval food was restricted, an imposed mortality of adults led to reduced larval competition and the emergence of more adults, and the same result was achieved by destroying a proportion of the eggs laid or by adding a barrier to reduce oviposition. An imposed reduction at any stage led to reduced competition and a compensatory increase (in fact, an over-compensation, because of the vigorous and wasteful competition at high densities). Analagous results were achieved by Watt (77), using *Tribolium* cultures as models for the study of population productivity. Simmonds (65) observed that if one species of parasite of the fruit fly is less effective than usual, this is offset by an increase in one or more of the other species. Schwerdtfeger (33) quotes numerical examples of the working out of compensation in populations of forest insects. The prerequisite for compensation is the presence of at least one limiting agent which is density-dependent in action.

Recent work has added to the many examples showing that the differential influence of weather may determine the effectiveness or otherwise of natural enemies [for examples see Andrewartha & Birch (5)]. Burnett (78) has pointed out the significance of such influences on parasite and host in facilitating the continued existence of both species. DeBach, Fisher & Landi

(79) consider that the climate in parts of California prevents *Aphytis* spp. from controlling the citrus red scale, so that the scale is most abundant not where climate is optimal for it, but where climate hinders the parasites. Climatic differences may also determine which biotic agents are the more important in different areas, as Lord & MacPhee (80) showed for the natural enemies of the oystershell scale in Canada.

Elsewhere (13) I have reviewed evidence for the conclusion that more complex and varied ecosystems provide greater compensatory resources and therefore greater stability of populations. For example, mixed forests are generally considered to be less prone to outbreaks of pests than are pure stands.

Density-Dependence

Density-dependence has usually been taken to include all density-relationships in which the mean effect per individual of the population is higher at high densities than at low densities. It usually includes even those cases in which there is a time-lag between the occurrence of a particular high or low level of density and the response to it on the part of the density-dependent factor, notably in the response of certain parasites and predators. This makes concise definition difficult and has led Varley (68) to maintain that parasites and predators should be excluded from the category of density-dependent factors. I think it better to accept the well-established usage, and not just because it would be confusing to change it. Admittedly the lag in the numerical response of natural enemies to the abundance of their hosts or prey often leads, for example, to a concentration of parasites and predators on the dwindling population. The relationship is temporarily the opposite of typical density-dependence, in that the proportion of enemies is greater than when the host or prey was abundant. Nevertheless, the effect is obviously the consequence of this former abundance. If more precision is needed, new names for the types of density-dependence will be necessary [cf. Nicholson's terms (12)]. Although it is convenient to think and write of density-dependent "factors," a factor may have this property at some times and not at others, or several factors may be jointly responsible for a density-dependent influence; therefore it is more accurate to think in terms of density-dependent "processes" (13).

The relationship of some factors with density is predominatly or completely inverse, i.e., the proportional effect per individual of the population is greater at low density than at high. Thus, while predators may kill greater numbers of a population when its density is high, the percentage killed may be less than at low density, in which case the density relationship is inverse (13). An example of a simpler type of inverse relationship is the shorter development period of certain lepidopterous larvae when they are crowded (81).

It is density-dependent processes which account for the regulation of populations. A regulator cannot operate as such unless it responds to some extent to changes in what is to be regulated. This idea is the same as that of

"feed-back" in cybernetics, though in the biological field there is no approach to precision except in certain laboratory experiments.

NATURAL CONTROL

"Natural control" can be defined as the process or complex of processes which keeps the numbers of animals, in a population not controlled by man, within the limits of fluctuation observed over a sufficiently representative period. It includes the idea of regulation, i.e., of control by density-dependent processes. Of course regulation does not proceed independently of surrounding conditions: usually, variations in weather and in many other environmental factors cause continual changes in the favourability or capacity of the environment, also in the activity and interrelations of all the species concerned, and hence in the level of density at which the regulating mechanism could put a stop to further increase of the population. Among these modifying factors is what Andrewartha & Birch (5) call "relative food shortage." Some examples are as follows: (a) *Thrips imaginis* Bagnall is greatly hindered during the summer in South Australia by the sparsity of the flowers it needs for feeding and oviposition. There is apparently no crowding or competition on these flowers, it is just that they are hard to find. (b) Milne (82) showed that the sheep tick has small chance of encountering a host when sheep are sparse, although there is no shortage of food if only the ticks could encounter it. (c) DeBach (83) studied a hymenopteron, *Lygocerus* sp., hyperparasitic on the larva of *Anarhopus sydneyensis* Timberlake which lives in a mealybug. *Lygocerus* seeks its host by probing in the mealybug with its ovipositor. When the mealybugs are abundant and most of them contain no *Anarhopus*, *Lygocerus* does not find its full quota of hosts. Hence its food supply is limited less by the absolute number of hosts present than by the percentage of mealybugs which contain one. The essential feature of relative food shortage is that the food supply is too sparsely distributed. Like the weather, this may have an important influence on the rates of mortality and reproduction, but in the same way at high and low densities of the population. Hence it cannot exercise natural control unless its action is linked in some way with a density-dependent process, e.g., by crowding effects between thrips on the flowers, or by damage to the flowers by the thrips, or by some influence of the ticks (e.g., as vectors of disease) on the numbers of sheep. Parasites and predators may suffer from sparsity of of hosts or prey which they have themselves brought about by reducing the numbers available; such an effect is density-dependent.

A possible cause of the decline of outbreaks which does not come within the category of density-dependent processes has been discussed by Franz (84). His theory is that since in an outbreak relatively small numbers multiply to high numbers by inbreeding in conditions of reduced selective pressure, deleterious recessive factors may become homozygous and so weaken the constitution of the insects. This may be a cause of the subsequent reduction to low numbers, especially when the normal selective pressure is renewed.

Since the ultimate cause is environmental variation, inducing corresponding changes in the constitution of the insects, such a decline in numbers is best regarded as analagous with straightforward fluctuations attributable to weather etc., and not as regulation.

We should not assume that a population is necessarily in process of being regulated all the time. Some populations may be so, others seem not to be. For the looser sort of control, it is necessary only that one density-dependent process or another should come into effective operation whenever the density becomes high, the critical density value tending to vary continually with environmental conditions. The density may remain below this level for considerable periods because of unfavourable weather etc., and during these periods there may be no factor operating with a density-dependence of much significance.

Effects of crowding and shortage.—Various ways in which crowding effects (i.e., effects of space-shortage) may reduce the rate of increase in laboratory cultures and bring it to a halt are well known. Crowding effects are perhaps rather uncommon in field populations. Shortage of food is probably more important, especially at times among predators and parasites. The fly *Anisopus fenestralis* Scopoli, whose larvae feed in sewage beds, is limited there by the amount of food available [Hawkes (85)]. As we have seen, the flies of carrion seem to suffer from acute shortage of food, complicated by intense intra- and interspecific competition (19, 54). I have already referred to the density-independent phenomenon of "relative food shortage." This must be distinguished from local food shortage, when a limited power of dispersal or the conformation of the environment puts some of the potential food supply out of reach. Clark (86) studied bettles of the genus *Chrysomela* in an area in Victoria, where they were introduced to control the weed *Hypericum perforatum* Linnaeus. When the beetles and their larvae had stripped the plants in open areas, the population suffered from starvation, in spite of the presence of their food plant in adjacent woodland. After defoliation the provision of food for the larvae depends on the beetles dispersing and laying eggs on fresh groups of plants, but although the beetles would feed on the plants in the wooded areas, they would seldom lay eggs there. The population was controlled partly by local food-shortage and partly by increased predation and frost damage because of lack of cover, operating in a density-dependent manner (at a low enough density of *Chrysomela*, there would have been no shortage of food or cover).

It was remarked earlier that dispersal is often density-dependent and that the surplus population may move into less favourable areas. This is a possible mode of natural control, which may apply, for example, to locusts.

Control by predators or parasites.—A population limited by natural enemies may be held at a much lower density than the environment could support in the absence of these controls. The fact that certain pests have been successfully and permanently controlled at an economic level by the introduction of insect parasites or predators shows that natural enemies

can limit populations in this way. As Taylor (87) has emphasized, there are less than 20 complete successes out of a great number of attempts. But in many of the attempts which have fallen short of economic perfection, the numbers of the pest have in fact been greatly reduced, as for example in some of the cases referred to in the *Report of the 6th Commonwealth Entomological Conference* (88). Apart from these results of artificial introductions, there is plenty of evidence of the importance of enemies in the natural control of many species [cf. Huffaker & Kennett (89); Thalenhorst (90); DeBach, Fleschner & Dietrick (26); Dean (91); Miyashita (92); Muma (93); and others (88)]. The fact that many pests, particularly in orchards, have become more numerous following the use of certain insecticides and fungicides is evidence that natural enemies had previously controlled these populations at a lower level, and in some of these cases more direct evidence is available [see review by Ripper (94)]. Of course, no one would claim that all, or even most, natural enemies are important in natural control. Good progress has been made in the development of field experimental methods of assessing the influence of parasites and predators [cf. DeBach (95); DeBach, Dietrick & Fleschner (96); Brian & Brian (97); DeBach, Fleschner & Dietrick (98); Huffaker & Spitzer (99); Dowden, Jaynes & Carolin (100); Franz (101)]. (Though simple in conception, these methods mark an important development: the analysis of population dynamics by field experiments.)

Complex control.—Some populations are apparently regulated indefinitely by a single factor, e.g., most of those pests which have been successfully brought under biological control. There are others whose regulation seems to be shared by several factors, either jointly or in different places or at different times. Different density-dependent factors acting together may be less effective than one of them could be alone. The advantage of a many-sided control is its greater reliability in face of changing conditions. Control by disease, which is notoriously sporadic, commonly alternates with control by other factors such as parasites, as Ullyett (102) observed in the case of the diamond-back moth in South Africa. Observations on the spruce budworm in Eastern Canada suggest that outbreaks depend on stand type, climate, and moth dispersal, while the subsequent decline is initiated chiefly by self-induced food shortage [Morris *et al.* (103)]. It has been suggested that "starvation and specific reactions to meteorological factors that drive large numbers of larvae from the trees prior to pupation . . . may decrease the spruce budworm population just enough to make it extremely vulnerable to the rapidly increasing populations of biological agents . . . " [Wellington *et al.* (104)]. The decline of an outbreak in the Adirondacks was observed by Dowden *et al.* (100, 105) and attributed to overwintering mortality followed by the attacks of parasites and birds. Some related species seem to be controlled primarily by parasites [Franz (106); Morris *et al.* (103)]. The numbers of the larch sawfly in Canada may be reduced by flooding or by parasites and small mammals; if a severe infestation continues for three or four years, food shortage sets in, and this enables the parasites and predators to gain

control [Lejeune (107)]. Franz (108) studied populations of a beetle (*Laricobius*) whose larvae are predatory on *Chermes* in Germany; they suffered from parasitism (and disease) most acutely when they were concentrated on the few remaining colonies of *Chermes* at the end of an outbreak. It seems to be true of a number of species that while parasitism and predation may be secondary to the termination of an outbreak, they play an important part in suppressing the population to a low level.

Natural control is complicated when important parasites or predators themselves suffer from natural enemies or competitors. O'Connor (109) and Way (75) showed that certain coreid bugs which damage coconuts are controlled by ants of the genus *Oecophylla*, but there are other ants which drive out *Oecophylla* under certain conditions determined by soil type and ground vegetation. Hence control by *Oecophylla* can succeed only if ecological conditions favour it against its competitors.

Nonregulation, or Natural Control by Chance

Davidson & Andrewartha (35, 36) carried out a sample-census of the number of *Thrips imaginis* in a garden in Adelaide, South Australia, throughout the spring and early summer for 14 years. The purpose of the work was to measure the association between abundance and the weather. The eggs are usually laid in flowers, and the nymphs and adults feed in the flowers of many species, but the nymphs go to pupate in debris around the plants or in the soil. Although they can develop and breed throughout the year, the period of rapid increase is the main flowering season in spring. After that, most of the vegetation dries up, and flowers are scarce except in watered gardens; the thrips suffer a very heavy mortality and remain at low numbers until the following spring. Andrewartha & Birch (5), in a full discussion of this work, draw the following conclusions: (*a*) Since 78 per cent of the variation in annual peak numbers was correlated with meteorological variations,

this left virtually no chance of finding any other systematic cause for variation, because 22 per cent is a rather small residuum to be left as due to random sampling errors . . . not only did we fail to find a "density-dependent factor," but we also showed that there was no room for one.

(*b*) Why does not *T. imaginis* go on increasing from year to year?

It just does not have time to do this, for the favourable season of the year, which is the spring, is invariably followed by an unfavourable period in the summer, when hot, dry weather (but not "density-dependent factors") knocks the numbers back.

(*c*) The dogma of "density-dependent factors" is unrealistic on at least two major counts: it ignores the fluctuations of *r* with time, which may be induced by seasonal and other fluctuations in the components of environment; it also ignores the heterogeneity of the places where animals may live. This empirical study of a natural population of *Thrips imaginis* has shown that if these two facts are recognized, it is not necessary to invoke "density-dependent factors" to explain either the maximal or the minimal numbers occurring in a natural population.

To take the last point first, there is no space to discuss whether some statements about density-dependent factors deserve the above strictures; the important point is whether density-dependence is essential to natural control or whether the well-recognized heterogeneity of environments in space and time can adequately account for it, at least in the case of *T. imaginis*. As to point (*a*), most of those who have written at any length about density-dependence make a distinction between the regulating mechanism and the general environmental conditions which determine, and vary, the level of density about which a population is regulated. The fact that most of the variation in numbers of thrips was correlated with variations in weather is not proof that there was no regulation. It is more to the point that the authors failed to detect any density-dependent factors, but they do not claim to have made a thorough investigation of this matter: the main project was not designed to test it. One would like to know, for example, whether there is any density-dependent element in the dispersal of adults when they are abundant on the flowers. The answer given to the question (*b*) offers us only a coincidence: that the time limit to the periods of rapid increase allows just the right number to be produced to offset, over a number of years, the mortality during the dry season. It seems extremely unlikely that this would happen, without the occasional regulatory assistance of a density-dependent process.

A second example of a species greatly influenced by physical conditions [Andrewartha & Birch (5)] is drawn from work by themselves and others on the grasshopper *Austroicetes cruciata* Saussure in South Australia. The eggs remain in the soil for the greater part of the year and hatch about the end of August. The nymphs and adults feed on the green vegetation, chiefly grasses. But by the time the adults have laid perhaps a quarter or a third of their full quota of eggs the herbage is dried up, so that they starve about the end of November. The density attained in different years varies from swarms to very few indeed. In some summers a high proportion of eggs die from desiccation, and if the grass dries up sooner than usual most of the grasshoppers may die before laying any eggs. The authors conclude that "the distribution and abundance of *A. cruciata* are determined largely by weather; there is no evidence for 'density-dependent factors'."

With the first half of this conclusion we can readily agree. We can also agree that *A. cruciata*, like *T. imaginis*, is saved from complete extermination by the existence of a few places which provide refuges in time of drought etc. But to explain why the great irregular increases and decreases from year to year add up over a period to approximately zero, we seem to have only two alternatives, density-dependence at some point in the population cycle, or pure chance. Not only does the former alternative seem the more reasonable, but from the account given by Andrewartha & Birch one can see several possibilities of density-dependent action. Mention is made of a species of *Scelio* whose larvae feed on the eggs of *A. cruciata*, and of a fungal disease of the eggs (though not seen in the area studied). Birds of various

sorts prey on the nymphs and adults in the spring, when they are feeding their young. In the drought year of 1940

there were scarcely enough grasshoppers to satisfy the birds, which congregated in the places where the grasshoppers had survived the drought, staying there until they had apparently sought out the very last grasshopper, with the result that a very high proportion of the insects was destroyed in this way.

One would expect that this extra mortality, imposed at a time when the population was already greatly reduced, might affect the abundance of the grasshoppers for years to come. But could it have been density-dependent? It might have been, for birds are long-lived and slowly breeding predators, and there is a good deal of evidence in support of the view that their abundance depends on the food supply [Lack (110)]. If the grasshoppers formed a staple part of their diet each spring, the abundance of the birds might depend partly on the abundance of grasshoppers in the previous few years. Thus although the birds have little effect on the numbers of grasshoppers in normal years, in the occasional years when these insects are at low density the birds may exert an influence which lasts for a long time afterwards. If this is so, the birds exert a suppresive type of delayed density-dependent action, comparable with the examples from forest entomology, quoted earlier. Thus there are possible density-dependent factors which have not been fully investigated, and the absence of density-dependence, which would be difficult to prove under any circumstances, has certainly not been established in this case.

The same considerations apply to other examples in which adequate density-dependent relationships have not yet been demonstrated. This failure, not suprising at this stage in view of the practical difficulties, is not sufficient justification for invoking the unlikely coincidence that the populations are controlled by chance.

COMPARISON OF VIEWS ON NATURAL CONTROL

The questions at issue between the different theories of natural control have recently been discussed by Nicholson (12), Andrewartha & Birch (5), and Thompson (111). Here only the essential points can be dealt with, and these very briefly.

Nicholson (12), writes, (a)

Populations are self-governing systems. They regulate their densities in relation to their own properties and those of their environments. This they do by depleting and impairing essential things to the threshold of favourability, or by maintaining reactive inimical factors, such as the attack of natural enemies, at the limit of tolerance. The mechanism of density governance is almost always intraspecific competition, either amongst the animals for a critically important requisite, or amongst natural enemies for which the animals concerned are requisites.

(b) Although population densities can be governed only by factors which react to density change, factors which are uninfluenced by density may produce profound effects upon density. This they do by modifying the properties of the animals or

those of their environments, so influencing the level at which governing reaction adjusts population densities.

Many conclusions based on this theory are dealt with in Nicholson's recent and earlier papers, and examples of their practical implications are discussed by Nicholson (11, 12) and Varley (34, 59, 68).

Thompson (111), holding with equal firmness to his earlier statements, denies that the above contentions (a) have general validity, and concludes,

If an organism does not multiply without limit this is because it is restricted by its intrinsic specific limitations in a world which is made up of an ever changing complex of other specific and limited entities. Climatic and edaphic factors are the basis of the environmental diversity, producing not only the fragmentation of habitats but a constant change in their character and location. This is the primary extrinsic factor of natural control in the sense in which I have used this expression. In extreme cases, where a chance conjunction of favourable circumstances has led to long continued increase, the induced shortage of requisites or the multiplication of natural enemies drawn in by the mass attraction of the host population, may reduce this population; but such cases are clearly exceptional. Populations, therefore, are not truly regulated but merely vary, although indefinite increase is unlikely and in the long run will become impossible.

Each makes minor concessions towards the opposite view. Nicholson writes that if environmental conditions are unfavourable at times, "density governance is merely relaxed from time to time and subsequently resumed, and it remains the influence which adjusts population densities in relation to environmental favourability." Thompson (see above) allows that the sort of regulation Nicholson postulates may reduce the population in extreme and exceptional cases.

Andrewartha & Birch (5) sum up their views as follows:

The numbers of animals in a natural population may be limited in three ways: (a) by shortage of material resources, such as food, places in which to make nests, etc.; (b) by inaccessibility of these material resources relative to the animals' capacities for dispersal and searching; and (c) by shortage of time when the rate of increase r is positive. Of these three ways, the first is probably the least, and the last is probably the most, important in nature. Concerning (c), the fluctuations in the value of r may be caused by weather, predators, or any other component of environment which influences the rate of increase.

They accept Nicholson's first category, shortage of resources, as a minor cause of limitation but do not look upon this as a density-dependent type of relationship. They accept his second category, natural enemies, as imposing a time-limit on periods of increase but make no fundamental distinction between this and the time-limits imposed by changes in weather etc. They hold therefore that the limitation of density is not density-dependent, a view fundamentally similar to Thompson's and opposed to Nicholson's.

My own view may be gathered from earlier pages. It may be that, in some way at present unknown, the heterogeneity of environments in space

and time can independently bring about natural control, but I cannot see a logical basis for this and shall remain unconvinced unless someone can demonstrate how it works out (or at least, for a start, how it could do so). On the other hand, there are many examples of natural control by density-dependent processes.

Personal viewpoints may be greatly influenced by experience of particular types of environments. In regions with a relatively equable climate favourable to insect life, biotic factors seem specially important and control by parasites and predators may be clearly in evidence. In less favourable climates with a "hard" season, physical factors seem more important in the determination of abundance. The same principles apply, but the emphasis is different.

NATURAL CONTROL AS A FOCAL POINT FOR RESEARCH

For many species it is relatively easy to determine the environmental conditions in which they are abundant, and those in which they are not. It is generally much more difficult to find out how their abundance is regulated. A good deal of the first sort of information has been gathered about the species most important to man, such as mosquitoes and tsetse flies. This work, although invaluable in its own right, has not enabled us to explain the natural control of these insects. It seems likely that the required facts will be very difficult to get. We need not only basic ecological work of the usual types, but also investigation aimed directly at finding and evaluating the regulating factors. In most cases this would involve a continuous census and table of mortalities due to different factors, in temporal order, over a number of successive generations [for short-term examples of this sort of undertaking see Varley (59) and Morris & Miller (112)]. To be adequate, the project would normally have to be repeated on at least a few populations of the species in different places.

Ordinary ecological work can give a very useful basis for practical control of a pest, and this stage cannot safely be passed over. Since abundance depends on general environmental conditions as well as on the regulating mechanism, the effects of the former must be taken into account at every stage. But a knowledge of regulation may open up new and more effective means of control, and prevent much wastage of effort; at the least it should increase the predictability of the effects of control operations. Simple examples which illustrate this principle may be found in the proposals of DeBach (113) for assisting the biotic controls of citrus pests.

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THE SYNOPTIC APPROACH TO STUDIES OF INSECTS AND CLIMATE[1,2]

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In recent years, unrest over the more static aspects of climatology has been even more widespread than usual [Leighly (1); Spreen & Manos (2)]. There is a current belief that neither static climatology nor its techniques are suitable for descriptions or studies of the essentially dynamic phenomena of the atmosphere, though they may be useful for textbook descriptions of average climates [Friedman (3)]. Consequently, although a form of static climatology may persist, an analytical, interpretive, and dynamic climatology is being developed to serve with it.

Properly applied, this development could change the course of insect ecology, which has suffered much from applications of static concepts in investigations of dynamic phenomena. For example, Andrewartha & Birch (4) recently attacked concepts underlying the belief that climatic factors may not control annual variations in the local abundance of insects, even when they limit diurnal, seasonal, and geographic distributions of such insects. Their comments may reopen controversies that surrounded Nicholson's earlier writings, though careful study of his most recent statement (5) suggests that part of any future debate may stem more from semantic than from other differences. If controversy develops, however, it is bound to be both lively and absorbing. Therefore, before it demands all their attention, new disciples and partisans should pause to consider one additional item. They will need different concepts of weather and climate.

If this statement seems too sweeping, refer to the literature of the past 25 years. Leads provided by Uvarov (6) in 1931 have helped to produce countless papers that stress the suceptibility of insects to climate, but remarkably few that show how this marked suceptibility is involved in population growth or decline. Even fewer suggest how it may be exploited in control operations. Since the climatic concepts applied in much of the recent literature are the same as those used in the past, it is clear that many authors have yet to benefit from the recent remarriage of climatology and meteorology. Continued application of the older, irrelevant concepts is unlikely to provide more suitable data for the resolution of future controversies. Since populations, weather, and climate are all dynamic, a static conceptual framework can foster only inept methods of attacking the problems involved.

In view of the situation outlined above, I believe this review will be

more valuable if it is limited to a discussion of how application of modern meteorological concepts could change the approach to ecological studies. A recent paper (7) outlined some approaches that were developed from these concepts, but the present review begins at a different point.

THE MICROENVIRONMENT

Methods of study.—In 1954 Smith (8) joined previous authors who had stated that proper understanding of the effects of weather on insects depends on an understanding of the microenvironment. The near vacuum that persists in the wake of such memoranda generally is ascribed to the following difficulties (8): (*a*) costly or nonexistent equipment, (*b*) bewildering complexity and variability in this environment, and (*c*) the appalling amount of data such complexity and variability seem to require for reduction to some semblance of order. The amounts of money and ingenuity spent on equipment for other tasks make the first apology suspect. The others seem more legitimate from one standpoint but, from another, remedies are revealed.

Long ago, meteorologists encountered bewildering complexity in the atmosphere when they tried to delimit the changing areas of different kinds of weather in order to chart their courses. They solved the problem by mapping everything as it was at a given instant and then repeating the process at selected intervals to observe any changes. In essence, this is the synoptic method, although we cannot adapt it to our purposes without borrowing heavily from the elaborations of synoptic meteorology, the basis of weather forecasting. It is important to remember, however, that the method itself is essentially a sampling procedure. It loses much of its strangeness when viewed as such. In insect ecology it can help to solve the problem of environmental variability if it is used with the concepts of fronts and air masses employed by synoptic meteorologists.

Many authors have warned that weather in the microenvironment differs from the general weather and therefore cannot be studied with instruments in the same place. Few have considered the fact that particular types of microweather are produced by characteristic kinds of general weather. Consequently, most field workers are not aware that variability in the microenvironment can be classified in terms of general weather types. The synoptic approach to microenvironments depends on such classifications.

Brooks & Kelly (9) have some comments on weather typing for ecological purposes that should be read attentively by all entomologists. With good reason they deplore the growing tendency to classify data in such vague terms as "a relatively warm, clear day" (cf. 10 to 13). In the past, I have sometimes recommended a form of this vague classification on the grounds that it was better than nothing. The validity of this criticism by Brooks & Kelly is shown, however, by the present unsatisfactory situation in entomological research. Such partial typing precludes comparisons of results not only between areas but also within an area. Consequently, there seems to be no suitable substitute for a system of weather typing that includes some ref-

erence to the synoptic situation in terms of the air-mass or frontal types in the vicinity in addition to the more usual reference to sky state.

Brooks & Kelly (9) add numerous other recommendations that are suitable for their purposes but not for those of field entomologists. Therefore, in place of their full procedure, I recommend a variation of a method developed in conjunction with my colleagues during the past 10 years. It has been implicit in much of our work, e.g., the mountain weather studies in connection with insect mortality in the Canadian Rockies (14) and has proved both successful and flexible. Indeed, as will be seen later, variations of it may be used not only during observations of insect development but also during the complementary studies of factors affecting population density and insect behavior. It combines classification of environmental variability by general weather types with reduction of environmental complexity by selection of habitat extremes. In fact, the success of the whole method depends on the occurrence of extremes in weather and habitat. The procedure is summarized briefly below.

Areas in which the insect occurs are examined to determine what different conditions they provide. The differences generally are associated with degree of exposure to sunlight, e.g., open-grown versus overtopped trees, but other standards may be used. When extremes have been determined, two areas are selected that differ as much as possible but are still within a few minutes travelling time of one another. This requirement is based on the assumption that lack of recording instruments forces the observer to use the same indicating equipment at both stations within a brief interval.

When the areas have been selected, standard screen equipment, a rain gauge, and any other general weather instruments the observer may possess are established at the more exposed station in a manner that conforms as closely as possible to standard meteorological practice. In accordance with this practice, records are taken from these instruments and states of the sky are noted. The required biological program is pursued with only this nominal attention to meteorological observations until preselected types of weather occur. When these types appear, an additional intensive meteorological program is begun in both areas.

Clearly, conditions in the various microenvironments of the two habitats will approach toward or diverge from one another and the standard station in different kinds of weather. Therefore, the most valuable information will be obtained if extreme types of weather are selected for the intensive observations. If the observer wishes, he may divide weather types initially on the basis of clear versus cloudy, wet versus dry, and cool versus warm days and nights for field recording purposes. At biologically significant parts of the season, frequent measurements of selected extremes among the microenvironments within the two extreme habitats are made throughout a few selected days of each weather type. Measurements may be made every few minutes or at any desired interval, since these days are given over to this part of the program. Since comparatively few records accumulate during any one day,

they can be examined quickly for apparent anomalies requiring further investigation. Thus, the observer often will find that only two or three days of one type are required during one part of the season, although additional observations during later parts of the season are advisable to allow for changes in solar elevation. It will be found that such a program requires little time and yields much more valuable information on how selected environments may vary than can be obtained from numerous continuous records collected with no consideration of the general weather.

Brooks & Kelly (9) imply that weather typing in terms of air masses or fronts should be done almost immediately. This is seldom necessary and often impossible in many field programs where the observer is isolated from meteorological services. The field worker will fare better if he visits a forecast office near his base before the field season begins and determines there the best surface criteria for distinguishing air masses of the general region (cf. 9). At the same time he may arrange to have synoptic charts saved for his return and can find out how much assistance he may expect during his survey of these charts. Once in the field, when he has selected the types of weather during which observations should be made, close attention to the timing of sky and barograph changes not only will prepare him for the onset of these types but also, in note form, will assist his later interpretation of the records [Brooks (15); meteorological references in Wellington (7)].

It is worth noting that when entomologists are able to give meteorologists good reasons why they need weather records in terms of weather types, such records are apt to appear (9). When each worker is able to state that his "clear-day" results occurred, for example, in polar continental air of a particular modification, comparisons of results obtained in different regions should lead to valuable generalizations.

The reader will find that Brooks & Kelly advocate much more frequent collection of records than I have suggested here. At this point, therefore, it is worth considering the fundamental difference between the interests of meteorologists and of most entomologists in the microenvironment. Lack of recognition of this difference has led workers in all ecological fields into difficulties.

Meteorologists are interested in the physical processes that occur in the lower layers of the atmosphere, and therefore they are willing to collect voluminous records if these will help their analyses. On the other hand, most workers with biological interests in the microenvironment simply want to know what differences occur there; for example, how temperatures of different leaves vary during different kinds of weather. Therefore, they are willing to forego all but the essential minimum of understanding of physical processes required for this task. Since they learn their methods from meteorologists, however, they often become involved in unnecessarily complex recording programs. Many complaints concerning the bewildering complexity of the microenvironment stem from this mutual misunderstanding of aims and requirements, and the ensuing difficulties often carry over into illustration of

results. Geiger (16), for example, despite his essentially ecological text, sometimes shows a meteorological bias in his illustrations. Attempts to follow his methods can lead an entomologist into difficulties (cf. 13), so that methods of presentation similar to some employed by Green (17) and Green & de-Freitas (18) are sometimes preferable.

The need for comparable results was advanced as an argument for considering large-scale weather systems in classifications of the microenvironment. Another even more cogent reason will arise if this environment receives the attention it deserves from economic entomologists. The microenvironment is the one portion of the total environment that can be most easily manipulated for the partial control of insect pests. Thus, schemes for taking advantage of the suceptibility of insects to climate are already within the grasp of economic entomologists [see also Uvarov (6) and Schimitschek (19) on local climates, and Buxton (20) on riverine species of *Glossina*]. There is no better reason for studying the microenvironment, in fact, and neglect of such studies may have been more costly than one might suppose.

For example, how often have applications of insecticides to field crops been necessary because ignorance of the microenvironment precluded changes in time of planting or in spacing or exposure of plants that might have produced crop climates unsuitable for pest species? How often have more recent pest problems arisen because supposedly improved cultural methods changed timing, spacing, or exposure to provide more favorable crop climates for pests? Such questions are unanswerable at present. Moreover, they are apt to remain so without basic studies to determine what the different crop climates are, how they may vary within different weather systems, and how such variability affects the insects that live on or within the plants. The research necessary to explore the various ramifications of this subject could supply future graduate students of entomology with degree problems for several decades.

There is a rich meteorological literature on the fringes of this subject to which meteorologists, hydrologists, and various agricultural scientists continue to contribute [Geiger (16); Schneider (21)]. Most of it, however, lacks the synoptic approach and frequently also lacks basic information that an entomologist needs. Therefore, since workers entering this field may have to find their own way along many of its avenues, a few suggestions are in order. In these suggestions, it is assumed that crop climates will be studied in conjunction with the activity or development of insects exposed to them, since there is little point to microclimatic catalogues compiled for their own sake.

As noted, basic determinations must include actual crop climates at different stages of crop development and in different types of weather. While these determinations are in progress, it also should be possible to determine effects of different planting or cultural practices on the various climates. Before useful conclusions can be drawn, it will be necessary to determine the maximum climatic change made possible by these practices that is consistent with other agricultural requirements and how this change may fare during

variable weather. In some regions the general weather could interfere so much with attempts to control crop climate that the method would be useless. Indeed, in any area some interference by certain weather types is to be expected. Such types remain to be determined. In this connection, if a method of climatic control seems successful in all but one type of weather, it will be necessary to determine how frequent such disturbances may be. The investigator should not assume that one season's records will serve. Climate may be as variable in its own way as weather (7) so that past records must be examined.

Those who wish to obtain the meteorological or climatological background necessary for these and other studies suggested in this review will find the references below valuable additions to the list already cited in an earlier paper (7). Two recent climatological texts stress descriptions of regional climates in terms of air-mass or cyclonic frequencies and frontal passages. The 1954 English edition of Trewartha (22) and the 1954 German edition of Flohn (23) should prove most valuable to workers in North America and Europe respectively. Linehan (24) has some comments on the air-mass calendar, and Frisby & Green (25) and Belasco (26) consider characteristics of air masses over the British Isles. Woodbridge & Decker (27) have applied a form of weather typing to summer frontal rains in Oregon, but their approach may not be suitable for ecological purposes. Both European and North American workers who wish to engage in historical studies for special purposes or in studies of population trends (q.v.) may find in major meteorological libraries the *U. S. Weather Bureau Historical Map Series* (for much of the Northern Hemisphere) and the catalogue of European large-scale weather patterns [Hess & Brezowsky (28)]. All workers with ecological interests should pay close attention to the excellent *Meteorological Abstracts and Bibliography* published monthly by the American Meteorological Society, Boston, Massachusetts.

Topographic influences.—There is an additional aspect of meteorology that is of some interest to those concerned with micro- or local environments. This is the effects of topography on air-mass and frontal weather. A knowledge of such effects is important to an understanding of how weather may operate in an area and is a useful aid in selection of extreme areas for study or in classification of weather anomalies for analysis.

Over much of the earth's surface, the migratory air masses and the frontal zones along their borders tend to approach an area from a rather limited range of directions. Since the weather they bring to the area depends partly on the types of barriers or gaps that lie in both their entrance and exit paths, changes in their directions of travel can increase or decrease such topographic influences. Thus, a frontal system approaching a station upslope tends to bring somewhat different weather than the same kind of front approaching down or parallel to the slope. If different kinds of fronts are involved, local weather differences attributable to differences in approach are increased correspondingly. High mountain ridges with complex pass systems

provide the most extreme situations, and the interplay of factors in such regions may have considerable entomological significance [Henson, Stark & Wellington (14)]. Nevertheless, recent observations suggest that even relatively small topographic irregularities coupled with quite small changes in the direction of frontal approach or in air-mass circulation may have surprisingly large effects.

This field is relatively untouched, because it calls for local observations in terms of the synoptic weather pattern instead of the usual descriptions of average differences between high and low ground. The synoptic approach stresses the fact that important differences occur on both the high and the low ground when the weather systems approach from different directions. The importance of these differences to insects has already been demonstrated (14).

A recent series of studies that has some bearing on this field has been reported by Schirmer (29, 30). He found that daily weather charts revealed the presence of streaks of heavier rain 5 to 15 km. wide and sometimes more than 100 km. long. When no rain fell, these general areas sometimes contained so-called "cloud streets," in which the clouds are commonly arranged in ranks. Schirmer was able to confirm several persistent directions among these streaks by close examination of annual rainfall means. He pointed out that some areas in their paths were wetter than simple topographic considerations would suggest. Since he has not gone very deeply into the various synoptic situations with which the streaks must be associated, he can give no very clear idea of the possible range of interplay between weather direction and topography. Nevertheless, most synoptic meteorologists would suspect that his observations could be duplicated in many parts of the world.

The consequences of these streaks for local vegetation were pointed out by Schirmer when he reported the presence of plants on sites where they should not occur ordinarily. The consequences for insects occupying sites just within and just outside the border zone of a streak may be more interesting. In apparently uniform situations, one group might receive much less solar heat and much more frequent precipitation than the other. There are interesting problems here for workers in good gliding country where the routes of any cloud streets generally are known to glider pilots.

Types of measurements.—Although there have been many comments on the slight value of standard weather instruments or records in many types of ecological research [cf. Smith (8); Andrewartha & Birch (4)], an additional fact is seldom emphasized. Many of the variables measured even in the microenvironment are not precisely those of greatest significance. Some of the more necessary information has not been considered, but its collection may require few or no instruments.

Consider, for example, precipitation measurements. Information obtained from the ubiquitous rain gauge is sometimes used, but there seems to be no entomological reference to a type of observation of the utmost importance; namely, the wetting times of different kinds of vegetation during different

types of rain, and their drying times in the different types of air that follow rain. Often, a knowledge of how much rain fell may be unnecessary, but information on the effects of the rain on the insect is always necessary. If the insect lives on vegetation, the observation above is required, but apparently it is never made. Properly developed, it should of course take into account not only the rainfall intensity and the different kinds of post-storm air, but also what sort of weather delivers the rain. The nearest approach to it in recent entomological literature is in Hughes' study of weather influences on numbers of insects caught with a sweepnet (31). In his observations he took into account the length of time since air within a grass layer was saturated.

There are few results from outside fields that are directly applicable to this problem. Linskens (32) measured the effects of an apple-tree crown on rain at the ground at different distances from the stem, but his results are primarily measurements of throughfall, though they are of interest to those concerned with insects that occur in such habitats. Similarly, many hydrologists [e.g., Trimble & Weitzman (33)] have determined how much rain comes through overtopping vegetation, and some have measured how much is intercepted, but part of what we need to know is how much is required to wet foliage. Stoltenberg & Wilson (34) approached this aspect most closely when they investigated interception storage of rainfall by corn plants. Perhaps workers concerned with dew measurement have most to offer [Duvdevani (35); Hirst, Long & Penman (11); Hirst (36); Schrödter (37); Steubing (38); Wallin & Polhemus (39); and see also Zikeev (40) for an annotated bibliography on dew].

After some preliminary observations to watch what happens during rainfall, it may be advisable to simulate plant surfaces for recording purposes. The various types of drosometers should provide logical starting points for any new designs. The Duvdevani gauge is comparatively simple (35). Schrödter's (37) thermoelectric method is necessarily less so, but is worth further consideration. Similarly, the recorder described recently by Wallin & Polhemus (39) may prove adaptable. Hirst (36) has developed a method of recording persistence of water droplets on actual plant shoots that at first seems ideal, but his apparatus may be too delicate for rough field use at a distance from a base. For a subsidiary problem, Blanchard (41) has the most recent description of comparatively simple equipment for recording raindrop size and the time of shower occurrence.

In the same vein, the various types of wind measurements should be reconsidered. When there is a real need for accurate measurements of wind speed and direction, more or less complicated instruments may be required [Broadbent (10); Johnson (42)]. Examination of the literature, however, suggests that interest at the micro-level may center more on how air currents of varying velocities act on or around the surfaces of obstacles. A few years ago a toy appeared that used a concentrated soap solution and a metal ring to produce hundreds of small, persistent bubbles at a time. One of the more practical applications of this idea has been suggested by Paulus (43), i.e.,

streams of bubbles should delineate air-current paths and turbulence around obstacles.

Temperature measurements are in a relatively satisfactory state. Thermocouples continue to be popular, partly because they still have fewer disadvantages than resistance thermometers [Mäde (44)]. They are best for psychrometric measurements in small spaces (45), although it is very difficult to ensure a constant supply of water at the correct temperature for the wet junction if continuous field records are required. Unger (46) has the most recent description of a regulated water supply [but see also Edney's most recent electrical hygrometer (47)].

Despite the growing popularity of thermistors, most current models have some disadvantages that make thermocouples preferable for field use. Most are not yet small enough to insert in plant tissues. A more important objection is that some thermistors are not completely interchangeable, so that if one is broken far from home, its replacement requires calibration against the old bridge before its readings can be trusted. This can be difficult in isolated field situations. On the other hand, broken thermocouples can be mended and, if necessary, recalibrated in the field with comparative ease.

One objection to thermocouples in the field is the maze of wires that soon obstructs an experimental area, especially where possession of recording potentiometers encourages wholesale dispersion of junctions throughout a plot. In many programs of measurements on selected days, such tangles can be avoided by attaching junctions permanently to sampling points with only enough additional wire for attachment to a lead from a portable potentiometer that has a compensating reference. The instrument is carried to the different points in turn for connection with individual junctions or with several at a time through a selector switch. This procedure also reduces costs when very small wire diameters are required, because heavier, less expensive leads can be attached a few inches from the small junction and carried back to the potentiometer. It also avoids the kinks and breaks common in long leads of fine wires. A variation of it was used by Henson & Shepherd (48) in their measurements of lodgepole pine needle temperatures.

There is an enormous literature on instruments designed for microenvironmental measurements. In addition to pertinent references cited by Wellington (45), Geiger (16), and Smith (8) there are extensive annotated bibliographies on many aspects of agricultural meteorology prepared by Schneider (21) for the West German Weather Service.

Behavior

References cited by Andrewartha & Birch (4) indicate the quantity of work on the effects of physical factors on insect behavior during the past 25 years. Even before Fraenkel & Gunn (49) provided a stimulus, a few workers had begun to move out of the laboratory to examine natural situations. Nevertheless, if one looks beyond fields covered by investigators concerned with *Glossina*, locusts, aphids, and some other insects that present problems

large enough to inspire attack by all possible means (4, 20, 42, 50), the balance between laboratory and field investigations is not very satisfactory. Glen (51) and Andrewartha & Birch (4) call for a more judicious balance between the field and laboratory and, in addition, stress the importance of behavior studies as a necessary part of any ecological investigation.

There is perhaps still too much emphasis on the effects of physical factors on development at the expense of investigations of the effects of weather on behavior. This statement does not detract from the findings of those who have studied development: it means only that there should be better balance between such studies and those on behavior. An insect must do certain things if it is to survive, let alone develop, and a little more attention to insect behavior will repay any investigator.

Weather may influence behavior in two ways. It may prevent or permit some act such as feeding, in which the insect must engage anyway, or it may evoke a specific response that occurs only under certain conditions, e.g., a changed reaction to light during overheating. Failure to distinguish between these influences may lead workers into difficulties, but truly adequate separation of the two has to be done in the microenvironment where the insect is. Perhaps some of the unbalance between studies of development and behavior and certainly much of that between laboratory and field investigations stem from the tendency to shun this fundamental level of the environment. Once again, the synoptic approach can provide methods of handling the complexity and variability found there.

Behavior may be classified as simply as weather, since there are only so many things one species can do. Such a classification can be used to great advantage in conjunction with the detailed microenvironmental observations described previously. Indeed, there is no justification for separating the two, since the best understanding of behavior or of the microenvironment is gained by observation of extremes. (Moreover, as already noted, mere catalogues of variations in the microenvironment are largely a waste of time and are too liable to fade gradually into hack work.) Therefore, in any behavior study, a series of intensive observations on behavior in extreme habitats should be combined with nearly simultaneous microenvironmental measurements there during each selected day. This combination will provide more valuable information on behavior in relation to weather than can ever be gained either by continuous observations with scant attention to weather or by the more usual sporadic observations made when time permits.

Green (17) used a version of the synoptic method to study the behavior and activity of myrmeleontid larvae and their prey in relation to sand-surface and pit temperatures. As in most previous work, his results are classified only in terms of sky states, but they present a clear picture of the effects of temperatures in various parts of ant-lion pits on the movements of the insects within them. His work is a good example of the value of the generalized synoptic approach when time is short.

More recent studies employing a version of the improved method recommended here have yet to be published. They have shown, however, that the

method is equally well suited for quantitative studies aimed at forecasting differences in both the timing and amount of such acts as oviposition or feeding liable to occur in populations in extreme habitats during different types of weather. Consequently, the method would be a useful addition to purely statistical studies aimed at forecasting changes in abundance, times of peak infestations, and the beginning or peaks of hatching in relation to weather changes [Pratt (52)].

Statistical treatment of groups is common in behavior studies, but results are interpreted most often in terms of an individual's reaction. Properly handled, such interpretations have a certain power, but they also are dangerous because of the all-or-none quality with which individual reaction often is incorrectly invested. Incorrectly assessed variability within and between individuals can invalidate predictions of behavior expected during different types of weather. Moreover, fluctuations in the population available to perform an act may have effects that far outweigh any influence of weather on individual performance. Johnson (42) comments on this during his discussion of factors affecting aphid migration.

Johnson (42) points out that migration in the individual behavioral sense and the collective aspects of numbers migrating are two separate phenomena. His chief complaint is that most workers have been too ready to interpret changes in numbers flying (which depend also on population changes) as if they depended mainly on individual flight behavior. Although his criticism is specific, it can be generalized readily enough. Its general object is one of the major dangers with which those who study the effects of weather on behavior must contend.

The apparent effects of approaching or receding fronts on insect activity (7) form one aspect of behavior in response to weather that has great predictive value but is still largely unexplored [Henson (53); Rainey (54); Lewis in (7)]. It is probable that frontal zones were directly involved in cases of increased insect activity or dispersal associated earlier with migratory barometric depressions [Uvarov (6)].

Whenever such relations are noticed, there is a tendency to search among the simpler and more familiar factors of the environment for an explanation. Thus, Rainey (54), after considering the fund of information on locust behavior, suggested that down-wind drift probably is the major factor in the observed collection of swarms in zones of convergence associated with the Intertropical Front. The data in his excellent paper certainly support this suggestion, but his purposes required no final decision on individual behavior patterns that might have been involved earlier. Similarly, Henson (53) considered the light reactions of *Choristoneura* adults and suggested that observed increases in flight activity ahead of convective storms probably result as much from rapidly decreasing light intensities under the advancing cold frontal clouds as from changes in other factors. On the other hand, Lewis's data [Wellington (7)] indicated that pressure fluctuations before and during frontal passages may be more important than temperature or moisture changes in increasing the activity of many insects.

These examples are drawn from data collected for very divergent purposes, so that it is unwise to draw generalizations from them. On the other hand, it should be recognized that even those data which suggest an effect of only one aspect of frontal weather on behavior afford no proof. In other words, we know that stimulation of insects by frontal weather occurs, and that this stimulation leads to greatly increased local activity near the time of frontal passage or to increased long-range dispersal, or probably to both. Therefore, if we wish, we can use this knowledge to predict changes in insect activity or the occurrence and direction of insect dispersal in relation to the passage and subsequent direction of movement of a front. In any recorded instance we are not sure, however, whether a single variable was more important than some unknown combination or whether other, subtler variables were involved. [For example, caged adults of *Choristoneura* kept in an illuminated, air-conditioned room sometimes rouse from near-torpor as a cold front approaches (cf. 53).]

It would be helpful if more observers used the synoptic method to accumulate suitably detailed trapping records. It would be even more helpful if laboratory investigators simulated the pre- and postfrontal changes that occur more or less violently in the vicinities of warm and cold fronts. Any such laboratory program must allow for the fact that some fronts are weaker than others, but estimates of the ranges to be expected in different regions could be obtained from records at local forecast offices. As an additional safeguard, it would be wise to allow for the effects of acclimatization, as Haufe (55) did during his studies of the effects of pressure on the flight responses of *Aëdes*.

Near thunderstorms and cold fronts, there are especially rapid fluctuations in atmospheric electricity as well as in atmospheric pressure. Stimulation of insects by changes in the electrical gradient has been suspected for many years (6, 56) but is still largely a matter for speculation in the absence of adequate records. Thus, Schuá (57) obtained good records of changes in the potential gradient but did not record probable accompanying fluctuations in pressure during his observations on honey bees. Despite this lack, the observations still indicated increased irritability of colony sentries and changed uptake of food by foragers during electrical disturbances. His more recent laboratory results (58) on the influence of fluctuating fields on the small mammal, the golden hamster, were more definite, because influences of other factors were eliminated. They showed that fluctuating fields corresponding to those of thunderstorms caused hamsters to move their nests from the part of the terrarium between the electrodes to an undisturbed part, whereas static and control fields had no effect. Since the potential gradient is a subtle variable that, like pressure, can act indoors as well as outdoors near frontal passages, Schuá's apparatus should be adapted to entomological needs.

Laboratory investigators interested in determining the physiological or chemical bases of insect behavior during frontal changes should maintain

contact with mammalian bioclimatology, because some experiments in the mammalian field may suggest possible lines of investigation. For example, Neuwirth & Hummel (59) used a solution of a recent blood-plasma substitute, polyvinylpyrrolidon, to observe weather influences on the ability of colloidal solutions to scatter light. Their figures show peaks of optical diffusion close to times of passage of cold fronts, in periods of horizontal shearing, or when the jet stream is overhead. Similarly, Caroli & Pichotka (60) tested the coagulation times of rabbit blood during different types of weather. They found decreased values a few hours before frontal passage, a sharp rise at or near the time of passage, and a drop to more normal coagulation times after passage.

Both laboratory and field investigators should consult some of the literature on medical bioclimatology before they become too deeply involved in experimental design. There are a number of papers in which statistical methods employed in the general field draw fire or are defended. Jessel (61) has an extensive criticism of most of the methods employed. Berg (62) believes that bioclimatological material is often statistically inadequate and inexact and emphasizes the fact that meteorological factors are not necessarily single elements but weather complexes. He examines several relations of birth, death, and specific diseases to frontal passages and confirms some. Geppert (63) takes analyses by other authors of the relation of lung embolism to different weather types, converts the observations to tetrachoric tables, and finds some significant relations by correct statistical methods. Gressel & Thalhammer (64) show a close statistical relation between urine sugar changes in diabetics and passages of surface or upper fronts and occlusions.

Sauberer & Silhavy (65) were in a better position than most to investigate death rates in relation to frontal passages. They worked with records from a home for the aged where all subjects were in the same place and were treated and fed alike. Nevertheless, these authors could demonstrate no relation until they determined the times of frontal passages over their own area to $\pm\frac{1}{2}$ hr., classified the frontal intensities, and arranged their biological data in the same time units as the weather records. After these steps, and mainly by means of the "n-method," they were able to show that unusually high numbers of deaths occurred most often on the day before frontal passage, almost as often on the day of passage, and least often on days between fronts. [Note: Those who prefer statistical methods discussed in terms of the problem to which they must be applied should consult the recent text on statistical methods in meteorology by Brooks & Carruthers (66)].

In one way or another, all the authors cited above make the point that the biological observations are the weak links. This is worth well-nigh continuous emphasis. The "after-thought" method, whereby biological investigation proceeds with no attention to the weather until some time after data are tabulated is bad enough in any type of ecological research: it is fatal to success in behavior studies, especially in those involving fronts. It is possible to align even sporadic weather records with tightly organized bio-

logical data obtained with that purpose in mind. It is impossible to use the most precise and detailed weather records with biological data collected with no special thought for the weather or its possible effects. This statement may help to sober those giddied by the apparent power of regression equations. Such equations are only as good as their data, and the quickest way to determine whether methods of collecting original records need reappraisal is to use an equation for the predictive purposes for which it was originally intended. Unfortunately, this is seldom done.

POPULATION PROBLEMS

Applications of the synoptic method and some concepts of synoptic meteorology in studies of the microenvironment or of insect behavior have been emphasized here because they have not been treated explicitly in previous publications. On the other hand, applications of similar concepts of dynamic climatology have been discussed before (7, 67) and therefore merit less attention here. Nevertheless, it is necessary to consider briefly how the concept of a variable climate may affect some investigations of population problems. It may affect experimental design in studies ranging from tests of relative hardiness of established and introduced species [Solomon & Adamson (68)] to analyses of complicated host-parasite population fluctuations.

The biological implications of climatic fluctuations have always interested investigators. Some recent work has dealt with the association in time between these fluctuations and changes in the abundance of native species that fluctuate violently in any event (7, 67). Pschorn-Walcher (69, 70) has taken a different approach by considering changes in insect distribution in relation to changing climate. He points out that Central Europe has experienced recent outbreaks of insects that formerly were destructive only in the southern or southeastern parts of the continent, and he relates these changes in distribution to the recent warm period. On the whole, the biological effects of this climatic fluctuation are better documented in Europe than in North America [Beirne (71)], so that a number of investigations similar to those cited by Pschorn-Walcher are open to North American workers.

Much has been written on the subject of food as a factor in the complex controlling insect abundance, but little attention has been given to the possible effects of a variable climate on the food plants. For example, Andrewartha & Birch (4) consider interactions between weather and food, but their main concern is with changes in the distribution and abundance of food during different seasons. Similarly, Atwal (72) is concerned chiefly with interseasonal differences, though he also considers the effects of changes in food quality as well as quantity in his excellent study of *Plutella*. Scharff (73) comes closest to the central problem of food and climate when he argues that *Melanoplus* populations are a direct product of their food plants and an indirect product of the weather which caused the plants to be there in their current condition, because he then considers annual differences in food quality and quantity. Recognition of the true variability of climate hitherto

concealed in the mean values of static climatology might lead to determinations of the effects of annual variations in food quality as well as quantity on annual changes in insect abundance.

Thus, future determinations of the chemical composition of foliage might be repeated during each of several consecutive springs or summers to allow for the effects of annual variations in climate. At present, many such determinations seem to be restricted to one part of one season. It is not surprising, therefore, that compositions thus established have a static quality that limits their further usefulness during population studies. (In the future investigations, weather types prior to the time of collection of material for analysis also should be considered, but this is simply a variation of the method discussed in previous sections.)

Investigators interested in the effects of climate on food composition should consult the recent *Proceedings of the Specialist Conference on Plant and Animal Nutrition in Relation to Soil and Climatic Factors, Australia, 1949* (1951). The several papers on vitamin or protein changes are not cited separately because they deal with effects of these changes on mammals, but they and others contain valuable background information.

Interactions between host insects and their parasites or predators is another field of study that might gain by application of the concept of climatic variability. The unfortunate consequences of the cleavage of the environment into density-dependent and independent factors are being offset by investigations such as Burnett's (74, 75), in which the different physical requirements of host and parasite are taken into account, or in which the effects of weather on the parasite are considered (76), but much remains to be done. One recent field and laboratory study, however, provides information on the distribution of parasitism in relation to climate that could be extended just as readily to encompass local fluctuations in host and parasite abundance [DeBach, Fisher & Landi (77)].

These authors (77) found that *Aphytis* parasites of the California red scale vary in their efficiency as controlling agents in three areas of California. In the mild coastal area, good control is achieved consistently. In the less mild intermediate zone, natural control occurs in some citrus groves but not in others, apparently because of different grove climates. In this zone, however, biological control can be obtained by periodic colonizations of the parasites. In the interior zone, where greatest climatic extremes occur, natural control is rare, and consistent biological control is impossible.

The authors discuss various effects of low temperature on the parasites, including a subtle one whereby populations might decline rapidly through excessive production of males if spermatozoa retained by mated females are killed by cold that does not kill the females. The point that interests them most, however, is that a host may not necessarily be most abundant in its optimum climatic zone. They suggest that if the true optimum for the host is also the optimum for the parasite, the host is apt to be scarce there. In a zone suboptimum for the host but even more so for the parasite, the host

may become relatively abundant because control by parasites may be neutralized by adverse climate. So far as it affects the distributions of host and parasite, such a difference in their climatic requirements is extremely important. It is equally important, however, when considered in terms of climatic variations in only one area, because of its possible influence on changes in the local abundance of a host. Since the harsher climates of the interior zones are, in fact, variable, the authors have at hand an outdoor laboratory for investigating changes in local abundance of a host and parasite with different climatic requirements.

These examples suggest how recognition of the dynamic aspects of climate might be applied in assessments of the relative importance of biotic factors that control insect abundance. Such an approach to population problems might lead to a more comprehensive theory concerning factors controlling distribution and abundance.

Field investigations that may lead to a comprehensive theory by virtue of their dispassionate approach and intensive studies are rare. Perhaps the best current example is the series by Morris and his associates on *Choristoneura fumiferana* (Clem.) in New Brunswick (e.g., 78). The study of climatic influences is an excellent subject for review here because it is based on concepts of dynamic climatology [Greenbank (79)].

New Brunswick lies in a major exit channel for North American weather systems. Fluctuations in the annual supply of these systems and periodic shifts in their storm tracks over the continent cause considerable annual variations in the regional climate. Greenbank (79) combined this information with a knowledge of the physical requirements of the larvae in his analysis of summer climates associated with the two groups of major outbreaks in this century.

Each group was preceded by a few years of summer drought, and Greenbank has shown that the extent of each varied with the extent of the drought. Thus, 1912 outbreaks were general to the Province, and drought during the larval period occurred throughout New Brunswick during the preceding years. On the other hand, 1949 outbreaks were confined to northern New Brunswick, and drought occurred only in the north during the preceding years.

Greenbank considers the regional climate in terms of the relative effects of weather systems crossing the region or passing it to the north or south. He discusses the marked differences in precipitation, cloudiness, and sunshine that result both from differences in the routes of the weather systems and from differences in the air masses involved. On this basis, he selects the combination of storm-track and air-mass types most apt to produce the dry, sunny weather favorable for larval development.

Greenbank had to distinguish between populations that might have increased locally during favorable periods and those that might have been established suddenly by moths transported from more western infestations by cold frontal storms. Since New Brunswick is in the weather exit channel,

it is peculiarly susceptible to such invasions. Farther west, fronts may be absent when moths occur or, if they are present, may travel subsequently over areas unsuitable for insect establishment. To determine the possible local importance of such transport, Greenbank trapped extensively and also used sampling records to reveal inordinate increases in the number of eggs in an area in relation to the number of resident female pupae previously found there (80). Unexpected increases in this E/F ratio, followed by the sudden appearance of a heavily infested area the next year, seem indicative of moth influxes. Since it is easy to determine both the number of cold fronts during the adult period and their paths in relation to sources of adult supply, it also should be possible to judge whether transport during one summer is frequent enough to be important to infestation intensities during the next.

This is another in the series of model studies by the New Brunswick authors. Throughout it, Greenbank has blended sound entomological and meteorological practices in a manner that merits the close attention of all who may wish to apply meteorological concepts in entomological investigations.

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INSECT MIGRATION

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The word "migration" is considered, for the purposes of the present article, to refer to movements of animals in a direction and for a distance over which they have control, and which result in a temporary or permanent change of habitat. In insects such movements may cover a thousand miles or more, but in other cases the distances are shorter and the lower limit of a true migration is difficult to determine. The numbers of individuals in a single movement may range from a few hundred up to thousands of millions.

In some groups of animals the word migration has been considered to apply only when there is a regular to and fro movement of the population between two areas at different seasons of the year; this point will be discussed more fully later, but at present all directional movements in insects are taken into consideration whether there is yet any evidence of a return or not. Practically all evidence refers to movements of winged adults: there are records of mass spreading of larvae of Lepidoptera, and young locusts move steadily across country, but apart from this it is doubtful if wingless insects undertake movements which could be considered as truly migratory.

Evidence of migration comes from various sources which may be briefly summarised as follows: (a) Observations by field naturalists of large numbers of insects flying steadily in one definite direction. Such flights may last for minutes, hours, days, or even for weeks on end. There may be only a single species involved, or the flight may include many species and even, at times, insects of different orders. (b) Very sudden appearances of winged insects in numbers in an area where they were not known to be present previously, and with no evidence of local breeding or emergence. A corollary to this is the sudden disappearance of insects when quite abundant, without any reason to suspect sudden death. (c) The presence of flying insects far out at sea, or on oceanic islands, and on the snow at high altitudes in mountain ranges. (d) In countries where insects have been under continuous study for many years, observations show that certain insects only occur at certain seasons, and cannot be found in any stage at other times of the year. Evidence of this nature has to be critically examined. (e) In recent years there has been evidence of migration from recaptures of marked insects.

As a result of accumulated observations of the above types, particularly in the last 50 years, we now know that migration occurs regularly in locusts, dragonflies, butterflies and moths, some Heteroptera, some beetles (especially the Coccinellidae), and some Diptera (particularly the Syrphidae); but there is scattered evidence that the habit is even more widespread than this.

HISTORICAL

Although the earliest reference to migration of locusts goes back to Biblical times (1) it was not until the first half of the nineteenth century

that anything in the nature of a scientific study developed. In 1845 De Serres (2) wrote a book on "Des Causes des Migrations de Divers Animaux," in which he devoted about 30 pages to migrations of insects. A little later Van Bemmelen (3) in 1857 and Hagen (4) in 1861 gave summaries of older records, the former chiefly of dragonflies, the latter chiefly of Lepidoptera. The first summary of information on migrations of Lepidopters in the tropics was by Piepers in 1890 (5). Between 1898 and 1901 Tutt (6) published a survey of migration and distribution of insects of all orders. In 1930 I published (7) a full account of migration in butterflies with a bibliography of about 800 references. In 1932 Fraenkel (8) produced a general survey of the migrations of insects of all orders. In 1942 I published, in collaboration with others, (9) a further discussion on problems of migration in the Lepidoptera with 750 more references, and at present I have in the press a second book (10) giving an account of migration in all orders of insects.

There have been thousands of papers published about locusts, dealing here and there with migration, but no summary relating particularly to this problem as a whole. Uvarov's discovery of the "phases" of locusts was first given in 1921 (11), and his book of 1928 (12) is a most important general introduction with 450 references.

Summary of Evidence in Different Orders

Orthoptera.—There is evidence of migration in certain genera of the Acridiidae (including the locusts) and slight evidence in some Locustidae (long-horned grasshoppers) and in some Mantidae.

The word "locust" has been used for many years for certain species of short-horned grasshoppers (Acridiidae) which have a habit of aggregating in enormous swarms and migrating over long distances. There are only six or seven species in the whole world which strictly come under this category, although severe damage is done at times by mass outbreaks of other Acridiidae. The "locusts" are as follows: *Dociostaurus maroccanus* (Thunberg) chiefly in the Mediterranean countries and Northwest Africa, and it does not appear to make migrations over very long distances; *Locusta migratoria* (Linnaeus) with various races or sub-species covering at times southern Europe, nearly all Africa, Madagascar, Southern Asia, the East Indies, New Zealand, and parts of Australia; *Schistocerca gregaria* (Forskål) chiefly in the northern half of Africa, and in Southwest Asia as far as Western India. *Schistocerca paranensis* (Burmeister) throughout most of South America, Central America, southern Mexico, and occasionally in some of the West Indian islands; *Locustana pardalina* (Walker) and *Nomadacris septemfasciata* (Serville) in the southern half of Africa; and *Melanoplus mexicanus mexicanus* (Saussure) [= *Melanoplus spretus* (Walsh)] which used to occur in enormous numbers in the Midwestern states of the United States.

No discussion on the migration of locusts can be attempted without first mentioning Uvarov's "phase theory," which he first put forward in 1921 (11). It is that with each "species" of migratory locust there is associated a

more solitary, more sedentary grasshopper, differing often in colour, structure, physiology, and behaviour, but actually a form or "phase" of the same species. These solitary forms had been previously known but considered to be distinct species. For example, *Locusta danica* Linnaeus, a solitary grasshopper, was found to be the *solitaria* phase of *L. migratoria*, one of the true locusts. It is now known that the "phase" in which the species occurs depends largely, but not entirely, on the degree of crowding to which the insects have been subjected. If eggs laid by a single *gregaria* female are divided into two batches, in one of which the resulting larvae are kept crowded together and the other in which each young hopper is removed to a separate cage as soon as it hatches, the former will grow into *gregaria* locusts, and the latter into *solitaria* grasshoppers. At first it was thought that all migration was in the *gregaria* phases, but more recent work (13, 14) has shown that long distance migrations are undertaken by the *solitaria* phase. Individuals of the solitary phase of *L. migratoria* are occasionally captured in the British Isles, and from measurements of these it has been established that they must have flown from the breeding ground in the Black Sea area, a distance of about 1500 miles.

The migrations of locust swarms appear to be more irregular and unpredictable than those of some other insects. This has recently been shown to be probably due to the fact that at the great height that many swarms fly, the wind speed is greater than the maximum speed of flight of the insects. Thus the determining factor in their distribution may be the wind direction and not any power of orientation in the locusts themselves (15, 16). There is evidence of a fairly large regular seasonal movement in *S. gregaria* in North Africa and Asia Minor. Here there are usually two broods per year, the winter breeding being more concentrated north of the desert belt. In the spring there is a southerly movement or drift, and the summer or "monsoon" breeding season is chiefly south of the Sahara. This is followed by a return northward to their winter quarters.

On the other hand an extensive series of migrations of *L. migratoria* in Central and Southern Africa between 1928 and 1934 showed no such seasonal pattern (17). There was an original outbreak centre in the middle Niger region of French Sudan in the first year, followed in the second year by a spread to the west, south, and east. In the two following years the eastward movement reached right across Africa and in the next three years extended to the south and southwest, reaching back again to the west coast in Angola. This seven years outbreak was roughly a clockwise spread round, but avoiding the wet tropical forest area of the Congo basin.

In the case of *M. mexicanus* in the United States it is now believed that the old Rocky Mountain locust (*M. spretus*) is the gregarious form and the present widely distributed *M. mexicanus*, the *solitaria* form of the same species. Some change in the environment, possibly the spread of large scale agriculture, has prevented the development of the gregarious form since the end of the last century. Although *M. mexicanus* is at times very destructive, it does not appear in the same devastating swarms as *M. spretus* used to.

There was some evidence of regular to and fro seasonal movements in *M. spretus*, and Riley distinguished between "invading swarms" which came from the northwest in July to September and returning swarms which flew to the northwest in May and June. An interesting aftermath of the great migrations of *M. spretus* in the past is the occurrence of millions of individuals frozen into glaciers in the mountains of Montana (18).

The general theory of outbreaks of locusts at present is that there are areas, often comparatively small, where breeding takes place slowly year after year, chiefly with the insects in the solitary phase. These areas are usually in districts of low rainfall. At intervals some change takes place, possibly an increased rainfall producing more luxuriant vegetation, which causes a greatly increased rate of multiplication, an increased habit of aggregation, the development of the *gregaria* phase, and finally a spread from the more permanent breeding areas over the surrounding areas, and often continuing for hundreds of miles. It is curious, and not yet properly understood, that the impetus of increased fertility and multiplication appears to persist for several generations, resulting in extensive damage in areas where the insects are not usually known. The problem of orientation and wind will be referred to again later on.

Lepidoptera.—The habit of migration is found in all families of the Rhopalocera or butterflies but is particularly frequent in the Pieridae, the Danaidae, and the Nymphalidae, and less common in the Lycaenidae and Hesperidae. In the moths, the habit is widespread in the Sphingidae and occurs frequently in the Noctuidae but appears to be less common in the Geometridae. In all night-flying Lepidoptera it is of course difficult to get direct evidence of migration, and it is interesting to note that in some day-flying moths (such as *Plusia gamma* (Linnaeus) and species in the small tropical family Uraniidae) there are more records of directional flights than in many of the butterflies.

As an example of migration in the Danaidae, *Danaus plexippus* (Linnaeus), ranges during the summer months over the greater part of the United States and southern Canada. In the autumn the butterflies move southward, at first in small groups but often congregating later into bands of many thousands, and finally reach the states bordering on the Gulf of Mexico and southern California. Here they settle on trees, very often close to the sea, and spend the winter in a state of semihibernation. In the spring the hibernating bands break up, pairing takes place, and both sexes move steadily north, laying eggs on the milkweed which is just appearing above the ground. Thus they repopulate the area deserted in the previous autumn. According to the latitude there are one, two, or perhaps occasionally three generations before the autumn movement sets in. The flight may be over 1,500 miles in each direction, and the same individuals which have gone south in the autumn return to the north in the spring, but there is no evidence that any of these return south for a second journey. In spite of this the same group of trees may be used year after year by the hibernating insects, as for example at Monterey on the coast of California. The most

northerly hibernating area known is in the neighbourhood of San Francisco (latitude 38°), but many thousands of butterflies fly southwards each autumn through Texas into Mexico, and what happens to these is at present unknown.

There are three races of *D. plexippus* in America, distinguishable by their colour and markings. The North American migrant form (*plexippus*) is found as far south as part of the Central American peninsula, and some of the northern West Indian Islands. The southern form (*erippus*) is found south of the Amazon and is probably also a migrant. Between the two in tropical South America is a race *nigrippus* which appears to be largely sedentary but has been recorded migrating in Venezuela. Intermediates are known between the North and Central forms, but not between the Central and Southern forms. The existence of these forms assists in fixing the limits of the migrations.

Vanessa cardui (Linnaeus) is a butterfly of world-wide distribution which, in the northern hemisphere, spreads each summer often to quite high latitudes, and retreats in the winter to dry sub-tropical areas. It breeds along the edges of the North African Desert in the winter, and in spring moves north across the Mediterranean into Europe, sometimes in millions. It regularly reaches the latitude of the British Isles, where it breeds during the summer, and in some years may go beyond this latitude to Iceland or Finland, where it has been seen beyond the Arctic Circle. There is some evidence of a return flight in the autumn. The distance from North Africa to southern Britain is about 1,500 miles, and on to Iceland almost another thousand. There is evidence of similar migrations over the whole width of Europe, and some records of mass flights across the mountains in northwest India. In North America the species appears to breed during the winter in the arid areas of northwestern Mexico and Baja California and moves northward in the spring, sometimes in hundreds of millions into the neighbouring states of the United States. The total amount of immigration and spread varies greatly from year to year, but it not infrequently goes northward into Canada, and north-eastward to the New England States, the Gulf of St. Lawrence, and even occasionally to Newfoundland. There is no evidence of winter survival anywhere in the United States, except perhaps in the extreme southwest, and no evidence of any extensive immigration through Florida or anywhere else along the Gulf of Mexico. There are a few records indicating southerly movements in the autumn, but they could not at present be considered as conclusive. The distance from Northwest Mexico to the mouth of the St. Lawrence river is over 3,000 miles.

These two species are typical of the summer migrations of butterflies into temperate and cold-temperate regions, and they closely resemble the movements of many migrant birds. The proportion of migrant species in such climates can be very high. In the British Isles, where the butterfly fauna is probably better known than anywhere else in the world, about 17 species out of a total of 68 are partially or completely dependent on immigration for their occurrence. In the United States 20 or 30 migrant species are

already known, but the list will probably be considerably extended with further study.

In the tropics butterfly migrations are on a very extensive scale, and in some areas are so frequent and conspicuous as to become sources of superstition to uneducated races. In Ceylon 70 out of a total of about 250 species of Rhopalocera have been recorded as taking part in what are locally known as "flights," which sometimes consist of snow-storm like masses of millions of individuals all flying steadily in one direction, sometimes for days on end. Their origin and destination are unknown, but their seasonal occurrence appears to be related to the monsoons, with a peak of occurrence at just about the beginning of the northeast monsoon which brings the rains (19, 20).

In tropical Africa there are many records of mass flights in a definite direction, chiefly relating to three species of Pieridae and one of the family Libytheidae. In the former family, *Catopsilia florella* (Fabricius) has been reported as a regular migrant, particularly in the eastern half of the continent from the Union of South Africa to as far north as Egypt, where it is an occasional migrant. In Southern Rhodesia it migrates in numbers to the southwest in December and January, and there is some evidence of a return flight towards the northeast about six weeks later (21). *Libythea labdaca* Westwood moves to the south in the Gold Coast and Nigeria about March and April, which is towards the end of the dry season, and there is some evidence of a second migration season later in the year (22).

In tropical America migrations occur on a gigantic scale, especially in the genus *Phoebis* (*Pieridae*). *Phoebis sennae* (Linnaeus) and *Phoebis statira* (Cramer) have been recorded in mass flights over most of tropical America. The former migrates each spring into the southern United States, and there is definite evidence of a return to the south in the autumn (23). In the Portachuelo Pass, in the coastal range of mountains in Venezuela, Beebe (24) has recorded countless millions of butterflies, belonging to over 250 species, passing through from north to south almost without a break from May to September every year. Day and night flying moths and many other orders of insects were also included in the flights. Nothing is known of their origin or destination, or of any return movement.

Similar migrations have been recorded in Australia, and one species of Hesperidae (*Badamia exclamationis* Fabricius) migrates along the east coast of Queensland, with evidence of a movement to the south in December and January (*i.e.*, spring and early summer) and a return flight to the north in March. It has been said, but without any exact evidence, that swarms of this butterfly cross the sea from New Guinea to Queensland (25).

In the moths very extensive migrations occur in many species of the Sphingidae, which are unusually large insects with a powerful flight. Out of 17 species of the family known to occur in Britain, 8 are complete migrants and seldom or never survive the winter in these latitudes. One of the most interesting species is *Celerio lineata* (Fabricius) which has one subspecies, *C. l. lineata* (Fabricius) in America, and another, *C. l. livornica*

(Esper) in Africa and Europe. The latter breeds in North Africa during the winter and invades Europe in small or large numbers in the spring. The larvae feed on vines and as a result of the immigrations there may be extensive damage to vineyards in Spain, Italy, and Southern France. In some years the moths fly still further north, and may reach the southern coast of England. Here however they are rarities, and in the past hundred years only about 1700 moths have been reported by collectors, of which 543 were captured in 1943; there have been many years without a single record. In North America there is a very similar state of affairs. The insects breed during the winter somewhere in Central or South America, almost certainly in the semiarid climate of the western coast. Thence they move north in the spring into the southern states where, as in Europe, they become a minor pest of vines. In a study of the past history of outbreaks of this insect, Grant (26) showed that there was statistically significant evidence for years of heavy or of light invasion to occur simultaneously on both continents.

Among the Noctuidae there are many known migrants of which historically the cotton leafworm, *Alabama argillacea* (Hübner), is of special interest as being the first case, outside Europe, in which migration was suggested for a moth. This explanation was put forward in 1847 by Gorham (27) to explain its curious seasonal occurrences, but it was not until about 50 years later that the theory became generally accepted. This species has some more or less permanent breeding ground in the northern half of South America, from which swarms migrate northward (some by way of Central America and Mexico) to invade the cotton belt of North America. There is a rapid series of generations following one another during the growing season and then the insects suddenly disappear, and cannot be found in any stage until the new immigration in the following year. There is very probably a return flight of moths to the south, but the insect is nocturnal and the number of skilled observers in South America is still very small, and so far no observations of such a movement have been brought forward. It is however a remarkable and unexplained feature that in the fall, just when one would expect such a southerly flight, moths may appear in the northeastern states and even in southern Canada—six or seven hundred miles north of the cotton belt—sometimes in such numbers round the electric lamps in cities as to be a positive nuisance. At the same period the moths may become a minor pest by piercing and sucking ripe fruits such as grapes and peaches (28).

A most remarkable case of migration in a noctuid moth is that of *Agrotis infusa* (Boisduval) the so-called "Bogong" moth of Australia. It has been known for over a century that the moths congregate in large numbers in caves and clefts in rocks at high altitudes in the mountains of New South Wales. Their numbers were so great that the aborigines of this area used to make journeys to the caves, where they stupefied the moths with smoke and used them as a source of food which is very rich in fat. Recently Common (29) has investigated the problem and finds that moths which have been breeding over a very large area in the lowlands during the winter, move up into the hills in late October and November (i.e., spring), and go into a state

of almost complete dormancy in caves and similar places. The population
reaches its maximum by mid-December, often as many as 1500 insects per
square foot of surface, and there is no decline in numbers until the end of
March (autumn) when all rapidly disappear. All the moths in this state of
aestivation are sexually immature. The particular caves investigated by
Common were in the Brindabella Range in the Capital Territory round
Canberra at heights of between 4000 and 6000 feet. A somewhat similar case
is found in *Chorizagrotis auxiliaris* (Grote) in North America, which has re-
cently been found to aestivate under stones on screes at altitudes of about
9000 feet in the mountains of Montana (30).

In the American Tropics there are two remarkable day-flying moths
Urania leilus (Linnaeus) and *U. fulgens* Walker both of which are regular
migrants. They are brightly coloured with long tails and are frequently mis-
taken for swallow-tail butterflies. *U. fulgens* ranges from Mexico through
Central America to Colombia and Ecuador, and records are frequently re-
ceived of large numbers crossing the Panama Canal. The flights appear to
be most frequent between March and September, and in some of the Central
American countries there is evidence of flights in two directions at different
times of the year (31). As long ago as 1857 Friedrich, as quoted by Van
Bemmelen (3), stated that this species (wrongly identified as *U. leilus*)
migrates every year in southeast Mexico, going to the north for about three
weeks in April. About five to six weeks later there is a return flight to the
south in smaller numbers, the females without eggs. This clear cut state-
ment has not yet been confirmed, but may well be correct.

Odonata.—We have several hundred records of mass flights of dragon-
flies moving steadily in one direction, but the information is usually so in-
complete, and so frequently the species is unidentified, that it is difficult to
interpret the data. In Europe the chief migrants are *Libellula depressa*
Linnaeus, *L. quadrimaculata* Linnaeus and *Sympetrum striolatum* (Charpen-
tier), but many other species are also involved. Of the 43 species of dragon-
flies known in Great Britain, 10 are partial or complete immigrants. In
1947 there was a very extensive immigration of *S. striolatum* into the south
of Ireland for two or three days, and it is almost certain that they had made
an overseas flight of about 500 miles from the Spanish Peninsula (32). There
are said to be quite regular annual migrations in a south-westerly direction
along the south-eastern shores of the Baltic Sea, (33) and mass migrations
are frequently seen further north in Finland (34, 35).

In the north-eastern United States, Shannon (36) has recorded regular
southerly or south-westerly movements of dragonflies in the autumn along
the shores of Long Island in company with migrating Monarch butterflies
(*D. plexippus*). The species concerned were *Libellula pulchella* Drury,
Anax junius (Drury), and *Tramea lacerata* Hagen. On one occasion he esti-
mated that 350,000 insects passed along the coastal strip within two hours.
In Argentina *Aeschna bonariensis* Rambur appears at times in countless
millions flying to the northeast, a short time before the arrival of a very

violent south-westerly wind locally known as the "Pamperio." None are seen to return (37).

It has been suggested, particularly in western Europe that the mass flights of dragonflies are associated with the drying up of their breeding grounds, but the evidence seems inconclusive.

Coleoptera.—There are quite a number of records of steady flights of beetles in one direction, but it is only in the Coccinellidae that the evidence is sufficient for us to begin to see any pattern. In some of the species of this family, there appear to be regular seasonal migrations between large breeding areas and smaller winter or summer quarters, where there is a dormant period. These resting places are often at high altitudes. In California *Hippodamia convergens* Guérin breeds during the summer in the coastal plains and lower valleys, and in the autumn the beetles fly a hundred miles or more to the mountains, where they hibernate under stones, dead leaves etc. at heights of about 5000 feet (38). In the spring they fly back to the breeding areas. For hibernation they often mass together in tightly packed aggregations of thousands of insects, and at one time these were collected and kept in cool storage, to be liberated during the summer when required, to control outbreaks of aphids. It was said that two men could collect over a million beetles a day. Similar masses of beetles have been found in the hills in the middle of the summer, but whether these are early comers, or late stayers, or some new phenomenon, does not appear to be properly understood. There is evidence of similar winter aggregations in mountains in many parts of the United States, but no general study of the migrations as a problem of its own has yet been made.

In many parts of Europe, North Africa, and Asia Minor species of lady beetles have been found in similar aggregations, usually in winter, but sometimes in mid-summer, often on mountain tops but also at sea level. There are many records of the sudden appearance of great swarms in England, often along the coasts, but sometimes well inland. In 1925 Marriner (39) collected records of great swarms of *Adalia bipunctata* (Linnaeus) which had suddenly appeared, and he considered that they had moved in a north-westerly direction from the south midlands to the extreme northeast of England (a distance of over 200 miles) and had crossed the central ridge of the Pennine Hills in two places. In Egypt in April 1939 Oliver (40) reported vast numbers of *Coccinella undecimpunctata* Linnaeus coming in from the sea on the Mediterranean coast. In addition to the millions flying there was a drift line for at least fourteen miles along the coast, with an estimated number of over 50,000 beetles per foot.

Other orders.—There are many observations suggesting migration among the Heteroptera, but the only one in any way properly studied is that of the small pentatomid, *Eurygaster integriceps* Puton, in Asia Minor, where it is a serious pest of barley. The insects pass one generation in the spring feeding on cereals in valleys, as for example in Uzbekistan, at a height of about 2000 to 3000 feet. The adults, engorged with food, leave the valleys about June and

fly up to a height of about 7000 to 8000 feet in the mountains, where they aestivate. In the autumn they become active again and descend to about 5000 feet, where they hibernate. Then in March or April, not having taken any food since the previous spring, they fly back to the level of cultivation, feed, mate, oviposit, and die. There is thus in each year a single life-cycle which has one feeding period, two periods of dormancy, and three periods of migration (41).

In the Homoptera flights of up to several hundred miles are known in the beet leafhopper, *Circulifer tenellus* (Baker), in North America (42) which, in spite of the small size of the insect, appears to be largely independent of wind direction.

In the Hymenoptera there is a remarkable case of something closely resembling migration in one of the burrowing wasp, *Sphex aegyptiacus* Lepeletier, which uses locusts as food for its young. In East Africa in 1939 the arrival of a swarm of *Schistocerca gregaria* was at once followed by the appearance of thousands of the *Sphex*, not one of which had been seen in the district in the whole of the preceding year. The wasps burrowed in the ground and caught, paralysed, and buried locusts from daybreak to sunset for two days. Then the locust swarm departed and within an hour not one *Sphex* was to be seen. All the wasps were females (43). Later several other similar cases were reported and one observer saw "black bees" in a mass the size of a tree flying in the midst of a swarm of locusts. There is no doubt that this species has somehow developed a migratory habit in order to keep up with the wanderings of its prey. It seems probable that in such a case any orientation is only a question of imitating the locusts, and is not determined independently by the wasps.

In the group of the sawflies, Tenthredinidae, there is also some evidence of migration and *Athalia rosae* (Linnaeus) has undoubtedly crossed from the Continent to Britain in enormous numbers, but at long intervals (44).

In the Diptera there are once more scattered records of mass flights in one direction in many families in many parts of the world, but at present the evidence is most definite in the family Syrphidae. In these hover flies there appear to be regular seasonal movements both in western Europe and in the eastern United States (and probably elsewhere). In the autumn of 1880 Eimer (45) recorded thousands of *Eristalis sylvaticus* Meigen and *Syrphus lavendulae* Meigen moving to the southwest up one of the valleys in southeastern Switzerland. Similar southward autumn migrations have been found recently to be of regular occurrence through many of the passes of the Pyrenees (46, 47, 48).

During the summer months great swarms of Syrphidae often appear on the south and east coasts of England but, as their origin is unknown, it cannot be assumed that they represent a return movement from the south. Quite recently southerly autumn movements of hover flies have been reported to be of regular occurrence on the east coast of England at Spurn Head (59). In America, Shannon (49) has seen autumn movements of *Eristalis* going southward along the shores of New Jersey. It is remarkable that the

observed migrations in Europe often include in the same flight species of two genera with widely different food habits in the larval stage: *Syrphus* which are aphid-eaters, and *Eristalis*, of which the familiar rattailed larva feeds on decaying vegetation.

In some of the migrations of Syrphidae there have been associated large numbers of other Diptera including species of the genera *Calliphora* and *Lucilia*, so that these groups (in the family Calliphoridae) must also be considered as possible migrants.

Discussion

It has been necessary to go into some detail in surveying the evidence of migration in insects because, unlike bird migration, the facts appear to be so little known, even among entomologists. Even with the greatly increased number of observers and observations in the past 20 years, we still have only a very superficial knowledge because of the enormous number of species in the class of Insecta and the difficulty of accepting field identification without specimens for examination.

We can now see, however, that the habit of migration is widespread among many groups of winged insects, although definitely more common in some families than others. In some species the migrations are as definite and as regular as those of many birds; in others, in spite of many observations, the pattern is still indistinct; in the majority of species the amount of information is quite insufficient for any theorising. The flights may include hundreds of millions of individuals and can extend over hundreds of miles. There are quite frequently flights in two directions at different seasons, in some cases the flight in one direction being in larger numbers than the opposite flight. In the temperate regions the movements are nearly always away from the equator in the spring and towards it in the autumn, and so are closely related to temperature changes. In the tropics the climatic relations are more uncertain, but in Ceylon and southern India they are definitely related to the changes of the monsoon.

In some cases breeding takes place at both ends of the range; in others the migrations are between breeding areas and areas for hibernation and aestivation. In some cases (as in the foothills of the Himalayas) the flights seem to be up hill at the beginning of hot weather, and down hill when the temperatures begin to fall. Mountain ranges of 10,000 ft. or over may be crossed, and overseas flights of hundreds of miles regularly undertaken. Each year millions of insects safely cross the Mediterranean and are not apparently confined to its narrower parts. In nearly all of the hundreds of mass flights of Lepidoptera that have been observed, the insects have been flying at the normal height for ordinary activity, nearly all below 10 ft. from the ground, with perhaps occasional stragglers over 20 ft. However, a few exceptions are known: *D. plexippus* has frequently been reported "as far in the air as the eye can see"; and during a very large migration of *Ascia monuste* (Linnaeus) in South America, numbers were reported by aeroplanes up to several thousand feet (60). There is evidence that butterflies may fly higher when

the wind is behind them and lower when there is a head wind.

The few flights of Diptera that have been observed have all been quite close to the ground, and in the autumn flights across the Pyrenees they are often just skimming the ground. In the locusts, on the contrary, although some swarms fly long distances like a blanket over the ground following all its contours, many others have been reported thousands of feet in the air, and it is common to see them like brown smoke high above, with the individual insect indistinguishable even with field glasses.

Having outlined the facts one comes to the problems of how and why? Of these perhaps the most fundamental is the nature of the orientation of the migrating individuals, as without orientation in a definite direction flights would be aimless and without any form of regularity.

There is no doubt that migrating insects are able to keep for day after day and mile after mile to a flight in a definite direction, in spite of changes in the type of country and its contours, whether they are on land or sea and in spite of many changes in the weather. This applies not only to day-flying migrants, but to the thousands of moths that fly by night, and to many day-flying insects that have to continue to fly by night when on a long sea crossing. The constancy of direction, at least in butterflies migrating near the ground, is very definite and remarkable. If they meet obstacles such as houses or trees they will nearly always rise and fly over them, rather than turn to left or right and go round. Migrating butterflies have been known to fly through railway tunnels and into windows at one side of a house and out at the other. Another interesting point is that if they are flying over the top of thick forest and come to a clearing they will descend at the near edge and rise again at the far side but do not fly straight over at the original level: the same habit has been noticed in butterflies crossing narrow valleys or canyons. On the other hand the direction of flight can be temporarily diverted by shorelines or by the contours of valleys, but in such cases sooner or later the insects will take up once again their correct orientation.

There are many cases of different species of insects all flying together in the same flight in the same direction, but also a few observations on different species flying at the same time and place in different directions. For example in East Africa in 1929 there was for nearly a month a north-easterly flight of *Catopsilia florella* (Fabricius) interlacing with a south-westerly flight of *Terias senegalensis* Boisduval (both Pieridae) while for two days of this month there was a thick flight of locusts, *S. gregaria*, going to the southeast. The three streams were all under ten feet from the ground, and the orientation of each species had no apparent relation to the others.

Many factors have been suggested as external guides to orientation: the sun, the wind, gradients of temperatures or moisture or barometric pressure, the earth's magnetic field, the movement of retinal images and so on. The sun (and the moon at night) can, I think, be ruled out as final guides unless we are willing to allow insects a sense of time which would enable them to allow for the movements of the sun, not only during the day, but for its setting in the west and rising again next morning in the east. Furthermore,

orientation takes place at night in spite of the complicated movements of
the moon and its periods of darkness. Also orientation is quite definite at
midday in the tropics when the sun is so near the zenith as to be practically
useless for horizontal orientations. It is, on the other hand, very noticeable
that migration, in butterflies at least, often ceases or is much reduced when
the sun is clouded, but so also to a possibly similar extent is normal non-
migrating activity. The same criticism can be levelled against the use of
polarized light (suggested as an aid to the "homing" of insects, but not so
far as I am aware, for their migration). The direction of plane of polarization
relative to the insect depends on the position of the sun and of clouds or of
the moon at night, all of which vary rapidly.

The wind direction is a much more likely factor, but in the Lepidoptera
at least I have come to the conclusion that the evidence is strongly against
any really obligatory association (50). Although we know of a few cases of
flight direction associated with a change of the wind (sometimes a difference
between morning and afternoon of the same day) there are hundreds of ob-
servations of flights continuing steadily in one direction day after day, in
spite of changes in wind direction, sometimes almost all round the compass.
For example in May 1937 *Pieris brassicae* (Linnaeus) was flying to the north
near London for about six days during which the wind was from the south,
southwest, west, east, and northeast. The same species was flying in the same
locality in July and August 1940 towards the south for over two weeks with
winds from the south, southwest, west, northwest, and northeast. *Ascia
monuste* was migrating to the south on the Atlantic coast of Florida in the
spring of 1938 and 1939 with winds from every point of the compass, but
chiefly from the southeast and east. Later in the summer of each year it was
flying to the north with almost exactly the same distribution of winds. Ex-
amples like this could be multiplied almost indefinitely. It is important to
remember however that all these flights of Lepidoptera were taking place
with most individuals less than ten feet from the ground.

In recent years Kennedy, Rainey, and others (15, 16) have stressed the
fact that as the maximum speed of flight of locusts in still air is not much
over ten miles per hour, and as swarms frequently rise to a height of 1000
feet or more, they will frequently be in conditions where the wind velocity
is greater than their own. As a result the direction of movement of the swarm
must be determined by the direction of the wind almost irrespective of
any effort of the insects to fly in any other direction. A detailed comparison
of swarm movements in *S. gregaria* with synoptic weather charts has
strongly supported this conception. In the Mediterranean and North Africa
the prevailing winds have a strong northerly component: in central Africa
there is a steady southerly component. Where the two streams meet is
known as the "Intertropical Convergent Zone," and it is along this belt that
the swarms tend to congregate. Changes in the latitude of the convergent
zone are associated with changes in the distribution of locust swarms, and
abnormal winds are often followed by abnormal swarm movements. Since
in any cyclonic air system the winds are blowing spirally into the centre, it

follows that there is also a tendency for locust swarms to be more frequent in areas of low pressure. This was noted in Egypt by Gough in 1915 (51).

Since rainfall tends to be more frequent towards the centre of a cyclonic system we get the interesting result that flight down-wind will bring the insects into areas where rain is more likely to fall. As locusts are in general inhabitants of semiarid areas their movement with the wind will automatically result in their reaching areas where vegetation is likely to be more luxuriant.

The orientation of locusts when near the ground is still not properly understood. In windy conditions they take off and land against the wind. as will most other insects. Sometimes there may be simultaneous flights in different directions at different levels. My own experience in East Africa showed no evidence of a steady relation between flight direction and wind, particularly if one remembers that if there is a prevailing wind direction and a prevailing flight direction, a relation of cause and effect is very difficult to prove unless changes in the one are reflected by changes in the other.

Locusts (and some butterflies) when migrating in a gusty wind have been observed to alter their body axes during a side gust, so that their direction of movement relative to the ground (track) was kept constant by varying the direction of their flight through the air (course) (43). This would indicate a definite means of orientation independent of any relation to the wind, and also of any image of the ground in the retina of the eyes. It is important to remember that the mechanism of orientation must enable the insects to resume their required flight direction after they have been diverted from it, either momentarily by gusts of wind, or by visiting flowers or small obstacles, or for longer periods by darkness or bad weather.

Gradients of temperature, moisture or pressure all fail to account for orientation, as none of these are constant at one spot. With the relatively short distance that an insect can move in one hour, the changes along the gradient obtained by such a movement (except possibly uphill) would scarcely be different from the average change experienced by staying still. Whichever direction an insect moves, and even if it does not move, in the early morning of a summer day, the temperature is likely to rise.

There is as yet no evidence of any influence of the earth's magnetic field on the orientation of insects (42), nor any suggested organ in the insect's body that could be supposed to be sensitive to magnetic polarity. It must be admitted that we are at present without any satisfactory explanation of the mechanism of orientation in migrant insects but, consider that, in spite of the known effect of wind on locust swarms, such an orientation exists as a fundamental factor in their migration.

A second most important problem is that of the so-called "return flight." For many years it was admitted that insects could fly quite large distances in a constant direction, but it was considered that these were only dispersal flights of a population overflowing from an overcrowded permanent breeding area. Flights of this type were thought to be different in origin and meaning from the true migration of birds, in which there was a to-and-fro

movement between breeding and nonbreeding areas. Heape (62) considered that true "migration" consisted of this double movement and was primarily due to a breeding or genetic stimulus, while "emigration" was the overflow from an overcrowded area, and was attributable to an alimental or feeding stimulus. In the latter there was no return flight. Heape only considered one insect, the butterfly *D. plexippus*, to be a true migrant: all others were "emigrants." When his book was published posthumously in 1931 we had very little data on return flight in migratory insects. Now we know of at least 20 or 30 species in which a return flight is well established. Since it is also known that within a species the flight in one direction may be dense and conspicuous, and in the opposite direction very thin and difficult to see, it is doubtful if it can be considered as proved that any migratory insect has no return flight.

This question is closely related to the problem of the evolution of the habit of migration. Even if many biologists do not accept all the implications of Darwin's Theory, it is difficult to believe that a habit can persist for thousands of generations, if every individual which shows it is lost to the species. This is the dilemma in which we find ourselves if we consider that insect migration is an overflow from some area, that none of the emigrants or their offspring ever return, and that the species is kept going only by the progeny of those that did not migrate.

Kettlewell (52) attempted to explain the dilemma on a genetic basis, and suggested that there might be two genes M and m, so constituted that MM were obligatory migrants, mm could not migrate, and Mm could migrate or remain sedentary according to external conditions. Big migrations would include MM and Mm; and Mm would, in the long run, have an advantage of flexibility over the two homozygotes. Later, however, (53) he said that if there was no return flight there would be a gradual depletion of the M gene in the central population.

A further interesting group of problems lies in the relation of population density to migration, particularly in view of the widespread idea that the mass directional movements so often seen are only overflows as just described. I have already mentioned Uvarov's "phase" theory, which is based on the fact that high population density can produce very definite effects on the morphology and activity of locusts. More recently it has been shown (54, 55, 56) that similar effects can be produced in Lepidoptera. At first it was thought that phases only occurred in migratory species, and that migration only occurred in the gregaria phase produced by over-crowding. Now it is known that locusts can migrate in the solitaria phase, and Long has shown (56) that some of the phase differences, particularly the darkening of colour, can be produced by crowding in the larva of *Saturnia pavonia* (Linnaeus). This species like others of its family emerges from the pupa in a sexually mature stage and usually pairs and lays eggs within a very short time. Further, it has no functional mouth parts and takes no food, and there is no evidence of any form of migration in its short life. Thus the connection between migration and phase is even less obligatory.

Some migrations undoubtedly start after an excessive build up of population, but even in a migratory species, great abundance need not result in migration. Two cases are known of enormous local abundance of the larvae of the pierid butterfly, *Glycesthia aurota* (Fabricius) (=*mesentina*), one in India and one in east Africa, when the food plants were almost defoliated. In neither was there any evidence of migration, and the resulting millions of butterflies were egg-laying on the spot. The complexity of the relation between aggregation and migration is well shown in *Danais plexippus*. In this, the larvae in summer are widely scattered over large areas feeding on plants of milkweed (*Asclepias*). In the fall the butterflies start to move to the south and gradually aggregate into larger and larger groups until, when they reach the southern states, there may be aggregations of many thousands of individuals. Here they lose the migratory instinct, but remain aggregated during their winter dormancy. In the spring the reverse change happens, they become active, lose their gregarious instinct, but resume their migrations back to the north.

The extreme thinness of population that can undertake quite definite migrations is perhaps best observed over the sea. In the Mediterranean I have seen a steady northward movement of *Vanessa cardui* when, with the closest watch, less than a dozen butterflies per hour were seen (57). The density must have been of the order of three or four per square mile. On the other hand, migrating swarms of the same species have been known to cast a shadow on the ground.

The number of insects concerned in migrations of different species varies very greatly from year to year. In the case of *V. cardui* it is sometimes almost absent from Britain during a summer, and sometimes very abundant. The same variation is also found in the United States. A statistical study of the years of unusual abundance in the two continents has shown (9), as in the case of *Celerio lineata* already mentioned, a significant tendency for the species to be either more common or less common simultaneously on both sides of the Atlantic. This suggests that the conditions (probably climatic) leading to unusually large migrations must tend to vary in the same direction over areas covering perhaps one-third of the earth's circumference. In nearly all migratory flights both sexes are present in approximately equal proportions, although there are reported cases where one sex only occurred. One has to be careful in arguing from the numbers of captured specimens as sometimes one sex is more easily caught than the other. Out of 633 desert locusts that I caught in east Africa 296 were males and 337 females: in a migratory skipper butterfly, *Andronymus neander* (Plötz) the captures were exactly 97 of each sex. On the other hand the *Sphex* migrating with the locusts in the same area were all females. A statement frequently made that only the female monarch butterfly migrates to the north in the spring is incorrect.

Many migrating insects, such as the locust, reach the adult stage in a sexually immature condition, often with a highly developed fat body. It is often in this pre-mature condition that the migrations take place. Later as the sexual organs begin to develop the migrations may cease. On the other

hand there are undoubtedly cases of migration in which the insects are completely mature and lay eggs during the flight. This has been observed in *Pieris brassicae* in England and in other pierids in Ceylon, East Africa, and Australia.

For about thirty years there have been various experiments on marking butterflies for recovery at a distance but it is only recently that results of any real value have been obtained. In view of the enormous number of individuals of insects of any one species, their short life (compared with birds), and the very low proportion which end up their lives in the hands of a collector, the chances of recovery of marked individuals is very small indeed. Early experiments were chiefly made by putting numbers or patches of bright colour on the wings of insects. The bright colours made the insect more conspicuous, but did not tell the captor what to do with his information. In 1938 we liberated several hundred butterflies about 25 miles north of London each with a splash of colour and a tiny printed label with the words "LONDON ZOO" and a number different for each individual (9) but there were no recoveries except near the point of release. A similar technique has also been used by Urquhart in Canada on monarch butterflies (58, 61) and he has been the first to get recoveries at long distances, in one case nearly 800 miles away from the point of liberation. This gives us the first absolute proof that single individuals are capable of flights over the distance which was considered necessary to account for the observed facts.

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RECENT ADVANCES IN VETERINARY ENTOMOLOGY[1]

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The advances during the last 15 years in our knowledge of the biology and control of arthropod pests of livestock and vectors of animal disease agents exceed those made in any similar period in past history. Before 1942 we relied mainly on rotenone, pyrethrum, the thiocyanates, and the arsenicals for control of lice, ticks, mites, biting flies, and cattle grubs. While effective against some pests under certain conditions, these materials were not practical for wide-scale use and did not meet the public demand for better insecticides. Today we have highly effective and low-cost insecticides such as DDT, lindane, TDE, toxaphene, methoxychlor, chlordane, and synergized pyrethrum for control of livestock insects. Their use has saved the livestock grower many millions of dollars annually and has benefited the consumer by making more and better animal products available.

Of almost equal importance to the development of the new insecticides are the contributions made to our knowledge of the biology and habits of several livestock insects and their transmission of agents of animal diseases. Many new ideas and approaches to studies on insect biology and control have been developed during the last few years. A good example of this is the unique method for the control of screw-worms by release of sterilized male flies over an area. The sterile males mate with the native females, but the eggs are infertile and thus reduce the numbers of screw-worms. Another example of new trends is the promising research with insecticides that can be given internally to livestock for destruction of external pests. These studies will be discussed in detail in the following pages.

Although great progress has been made in the use of insecticides, two disturbing factors have arisen to cause worry as to the future efficiency of chemical means of control. The first is the increasing and widespread development of resistance of insects to insecticides, particularly to the chlorinated hydrocarbons. House flies have developed such a high degree of resistance to DDT and related materials that satisfactory control is impossible in most areas. Organic phosphorus insecticides have so far performed in a creditable manner in controlling house flies, but there are indications that these chemicals may eventually fail. As yet no reports on resistance of horn flies, horse flies, deer flies, stable flies, sheep keds, or lice of livestock have appeared.

Ticks have become highly resistant to DDT, BHC, and toxaphene and other chemicals in certain areas in South Africa and Australia [Whitnall

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[1] The survey of literature pertaining to this review was completed in April, 1956.

et al. (1); Fiedler (2); Hitchcock (3)]. No doubt, other livestock insects will become resistant to insecticides, and it is readily understood that this stimulates extensive research to develop new materials. For detailed information on the genetic and biochemical aspects of resistance of arthropods to insecticides the reader is referred to an excellent review by Hoskins & Gordon (4).

The other disturbing influence is the finding that most of the chlorinated hydrocarbon insecticides applied to livestock are absorbed by the skin and stored in the fat, meat, or milk. A vast amount of research has been done to determine the amounts of various insecticides thus stored. In the United States the amounts of pesticides that are tolerated in edible food are regulated by law. The passage in 1954 of the Miller amendment to the Food, Drug and Cosmetic Act has made it necessary to intensify research on insecticide residues in animal products.

In the last few years several hundred papers have appeared that in one way or another deal directly with or relate to insects of veterinary importance. This large number is understandable when one recognizes that many hundreds, perhaps thousands, of species of insects attack animals. Many of the papers are on the taxonomy of single species or groups of genera and species. For the purposes of this paper it is not practical to refer to each of the papers. Furthermore, numerous species attack man as well as animals and reports on the taxonomy, biology, and control may refer primarily to the insect as it relates to man and may therefore not be directly applicable to the present scope of this paper.

It is the purpose of the writers to review numerous publications and to discuss important and significant trends in research on insects affecting animals. Complete coverage is not contemplated and many papers reporting excellent research will of necessity be omitted. Because of Fuller's (5) review of work on ticks and mites in relation to animals, no reference will be made to these important arthropods. Neither will house flies as livestock pests be discussed, since Lindsay & Scudder (6) reviewed the role of these flies in relation to disease of both man and animals. It is believed the reader will understand that the scope of veterinary entomology is indeed enormous and that there is increasing interest and a growing number of research papers.

BITING FLIES

Biting or bloodsucking flies of several families, numbering hundreds of species, are the most important livestock insect pests. Some species of biting flies are found wherever man and animals live.

Glossina.—Biting flies of the genus *Glossina* (tsetse flies) are restricted to Africa south of the Tropic of Cancer. These flies, infesting about $4\frac{1}{2}$ million square miles of land, are enemies of both man and animals, and have been a serious obstacle to the development of tropical Africa. The livestock industry has fared badly in these areas, since the trypanosome causing nagana is transmitted by these insects. At least 21 species of *Glossina* may feed on cattle and are capable of transmitting the agent of this disease.

Numerous papers on various aspects of the biology, control, and disease transmission of tsetse flies have appeared during the past 25 years.

Research on the control of tsetse flies has been directed along many different lines of attack, and against all stages of the pest. Control of pupae in breeding places by means of parasites, mechanical devices, and insecticides has been studied. Fly traps, screens, adhesive surfaces, reducing or expelling game animals (thus depriving the flies of food supply), clearing of thickets and/or modification of cover (according to the habits of the particular species), and introducing appropriate agricultural practices as well as application of insecticides directly on cattle and against the adult fly on an area basis have also been studied.

Buxton (7) has critically reviewed most of the literature in his comprehensive book on the natural history of tsetse flies. This book brings together a vast amount of knowledge and coordinates laboratory and field experiments and observations. It includes discussions of the anatomy, taxonomy, distribution, physiology, food, and relationships to the environment as well as a long chapter on control. The last two chapters deal with the importance of entomology to the epidemiology of the human and animal trypanosomiasis. Twenty-seven pages of references up to 1952 are listed, including numerous mimeographed papers and reports that are not readily available.

Another excellent review on trypanosomiasis and control of tsetse flies by chemical means in Zululand is by du Toit (8). This 70-page treatise deals with the distribution of nagana and its importance to the livestock industry, the bionomics of *Glossina* species in Zululand, and survey methods. The report on application of DDT and BHC with aerial sprays and aerosols and hand-dusting equipment for adult control is especially good. The methods of chemical control are well illustrated, and there are maps on distribution of various species of adults and of the concentrated larval breeding beds.

A detailed entomological study of *Glossina palpalis* (Robineau-Desvoidy) in northern Nigeria by Nash & Page (9) yielded much information on populations of the fly, its flight range, suitable macro- and micro-climates during dry and hot seasons, and longevity. Evidence derived from recovery of marked specimens indicates that females live up to 60 days. The authors estimate that wild female flies will live from six to fifteen weeks and the males from four to eight weeks according to the season. Jackson (10) has discussed marking adults of *Glossina* for release and subsequent recapture in population studies. A simultaneous census of two species of *Glossina* and animal hosts made along the Kenya shore of Lake Victoria by Glasgow & Wilson (11) indicated that an average of 291 flies fed daily on each animal, causing it to lose 17.5 gm. of blood per day.

Efficient methods of rearing insects in the laboratory are important in entomological work. Nash (12) studied fertilization of *G. palpalis* in the laboratory in order to rear large numbers at reasonable cost. It was found that females two days or six to ten days old were difficult to fertilize. Fertilization was greatest among three-day-old females. Males seven to eight

days old were more virile and produced a higher per cent of fertilization than younger or older groups. In large-scale breeding it was most efficient to place three-day-old females with twice as many males seven days of age.

Glover *et al.* (13) reported on the extermination of *Glossina morsitans* Westwood at Abercorn in northern Rhodesia. Fire exclusion with the object of allowing woody vegetation to thicken so as to make it unfavorable for the flies was begun in 1936 and continued to 1946. Catches of flies were reduced by one-third. In addition, shallow valleys forming the headwater of streams and harboring concentrations of flies were cleared of trees. The flies were greatly reduced, and cattle can now be kept free of trypanosomes in the Abercorn area.

The effect of DDT and BHC sprays on the parasites of tsetse flies in a Zululand fly-eradication campaign was studied by Fiedler *et al.* (14). At first it appeared that the spray campaign did not severely affect the parasites, but later it was found that both insecticides reduced the parasites before tsetse flies were eliminated. It was concluded by these authors that it is unwise to attempt eradication unless rapid and complete elimination of the fly can be achieved by insecticidal or other equally effective means.

Burnett (15) reported on an experiment in Tanganyika Territory designed to eliminate two species of *Glossina* by means of bait cattle sprayed with DDT. It is estimated the ratio of sprayed cattle to large game was no more than three to one. *G. moristans* was reduced by 99.5 per cent and *Glossina swynnertoni* Austen by 92.5 per cent. It was surmised that this method might check the disease in an area.

Hocking *et al.* (16, 17, 18) describes airplane spray (aerosol) experiments over large areas in the central province of Tanganyika against adults of *Glossina*. In the first experiment seven applications of 0.25 pound of DDT per acre over a $3\frac{1}{2}$-month period reduced adult populations 95 per cent or better. In the second experiment BHC was used. It was less successful than the first one because of a variety of factors, but probably the rainy season and full leaf forest canopy reduced the spray effectiveness. In the third experiment, conducted in an isolated area where infiltration of flies was unlikely, seven applications of DDT at 0.25 pound per acre at fortnightly intervals reduced the flies almost to extinction.

Tabanids.—Tabanids are among the most important bloodsucking insects affecting man and animals. They not only are severe pests but transmit several disease agents. The many hundreds of species are receiving much attention on a world-wide basis from a taxonomic viewpoint. The need for organizing the mass of information on many species, and preparing keys for their identification, has stimulated capable workers to attack the problem in several countries.

Oldroyd's (19) monograph on the horse flies of the Ethiopian region is an outstanding contribution to the knowledge of classification of this group. Besides the systematic study the volume contains information on the habits,

early stages, morphology, and transmission of disease. The 31 maps show distribution of species groups for 147 species.

Two recent publications by Philip (20, 21) add to our knowledge of North American Tabanidae. The earlier work contains notes and keys to the genera and species of Pangoniinae exclusive of *Chrysops*. It provides keys for the subfamily Pagoniinae in the entire continent which is far better than arbitrarily separating the tabanid fauna north of Mexico from that of the Mexican, Central American, and Caribbean areas. The later paper presents similar notes and keys to the genus *Chrysops*.

The classification and distribution of Tabanidae in Australia has been studied by Mackerras (22, 23). His papers represent a thorough systematic overhauling of the groups, including study of the genitalia together with other characters.

Basu *et al.* (24) published on the distribution of tabanid flies in India and their relationship to surra [*Trypanosoma evansi* (Steel)], a disease highly fatal to equines, camels, bovines, and dogs. They list 75 species known to occur not only in India, but in Pakistan, Ceylon, Burma, and the Persia-Baluchistan-Afghanistan area. Distribution maps of most of the species are given. Seven of the species transmit surra. *Tabanus rubidus* Wiedemann is undoubtedly the principal vector in India.

The effect of light on the biting activity of *Chrysops silacea* Austen in the forest at Kumba, British Cameroons, was studied briefly by Crewe (25). He concluded that there was no close correlation between biting activity and measured light intensity.

Fairchild (26) studied the arboreal habits of tabanids in Panama and found that several species preferred the upper forest canopy to ground level. Several groups of these flies seem to prefer this upper habitat. This is not surprising in view of the many mammals living in a tropical arboreal element. Lumsden (27) presented data on the biting habits of several groups of insects, including tabanids, in the forest canopy in Uganda. Duke (28) studied the biting habits of *C. silacea* by trapping them at various levels above the ground. Most of the flies were caught between 11 a.m. and 3 p.m. at heights of 28 and 92 ft.

Information on the life history is available for comparatively few species of the tabanids. The immature stages develop slowly, the time required to attain maturity ranged from a few months to over two years. Jones (29) summarizes a large amount of research in Florida on the distribution, seasonal occurrence, abundance, life history, larval habits, and natural enemies of tabanids. Specimens reared to maturity from field collected larvae and eggs showed the various species had an incubation period of four to six days, larval period of 220 to 807 days, and pupal period of five to fourteen days. Larval habitats were usually in swamps and around lakes, ponds, and streams, but a few larvae were found in rather dry places. Parasites and predators of all four stages of tabanids were recorded. Preliminary work on

the biology and distribution of 30 species of tabanids in Mississippi was reported by Lewis & Jones (30). Blickle (31) lists tabanids from New Hampshire, and Fairchild (32) lists those from Connecticut. Knutson *et al.* (33) list 37 species from New England.

Research on control of tabanids has consisted mainly in the use of insecticides on an area basis to destroy adults and of sprays to livestock to protect them against attack by the flies. Neither of these two methods have been effective or practical. Likewise, insecticides for control of larvae have not shown much promise, because the larvae are usually distributed over such large areas and embedded in soil or under water where insecticides do not reach them.

Brown & Morrison (34) conducted large-scale aerial spray tests in Canada to control adult tabanids. Lindane at 0.5 pound per acre eliminated *Tabanus* and *Chrysops* for two days in open situations, but in wooded areas caused only an 82 per cent reduction. A dosage of 0.25 pound per acre gave poor results even in open forest. DDT at dosages up to two pounds per acre gave temporary reductions of 53 to 100 per cent, but there was no evidence that the residue on vegetation was effective. Dieldrin at one pound per acre gave poor results. This work is of interest because of the unique methods of evaluating results. The population was assessed from landing rate on small tents made of black cotton cloth. Further checks were made by tethering flies to branches in the sprayed area by means of yellow nylon thread tied around the abdomens. One must conclude from this and other aerial spray work that control of tabanids on an area basis is difficult and expensive.

Roth (35) evaluated 16 insecticides against *Chrysops discalis* Williston by topical applications of measured dosages on collected flies. Lindane, the most effective insecticide, gave 100 per cent kill at 0.05 μgm. per fly, and the LD-50 was approximately 0.035 μgm., which was about $\frac{1}{13}$ that for toxaphene. Surprisingly enough, EPN, malathion, Diazinon, and other organic phosphorus compounds were less effective than most of the chlorinated hydrocarbons.

Roth *et al.* (36) evaluated 258 synthetic organic compounds as repellents in the laboratory using mice as the host animals and starved *C. discalis* as the test insects. Only a few compounds showed promise as long-lasting repellents. Tests in the field with five of the best materials applied on calves against mixed populations of tabanids showed protection of 2 hr. to 3$\frac{1}{2}$ days. Pyrethrum and allethrin sprays did not protect animals for more than three days even when used at high concentrations. Field tests with sprays applied to livestock to protect them against tabanids are reported by Goodwin *et al.* (37), and Bruce & Decker (38, 39). They showed pyrethrum preparations were practical and effective against tabanids in the midwestern and eastern United States.

A valuable contribution to the application of insecticides on cattle for the control of biting flies was made by Bruce (40), when he designed a walk-through treadle-sprayer which permitted the cattle to treat themselves

when going to the barn or to water. A mist of insecticide, usually a pyrethrum concentrate, is sprayed on the animals.

Outbreaks of vesicular stomatitis, a disease of livestock, particularly of hogs, seem to be associated with the eating of raw garbage. This disease is known to have spread rapidly from a center of infection making mysterious jumps of great distances where roads and other means of travel between farms are lacking. This has suggested that the virus may be insect borne. In studies on the laboratory transmission of vesicular stomatitis Ferris *et al.* (41) developed a method of using embryonated chicken eggs for use as both the infective and susceptible hosts. Twenty-eight species of biting Diptera were tested. Six species of *Tabanus*, three of *Chrysops* as well as some mosquitoes and the stable fly, were able to transmit the virus under laboratory experimental conditions. The transfer of the virus of vesicular stomatitis appeared to be mechanical, i.e., carried on the mouth parts of the insects. In field tests when horse flies, stable flies, and mosquitoes were exposed to the virus and allowed to bite two cows, transmission could not be demonstrated.

It seems appropriate to call attention to the valuable paper of Day & Bennetts (42) on specificity in arthropod vectors of plant and animal viruses. There is increasing evidence that any pathogen can be transmitted by more than one species of vector. The occurrence and significance of specificity in insects are discussed. The data on pathogens of medical and veterinary importance are summarized in 48 pages which includes 9 rickettsiae and 28 animal viruses that may be transmitted by insects. These records list species of insects that have been proved in laboratory and field experiments to be vectors and the species that have failed to transmit the disease. In addition information is given on the viability of the virus, multiplication within the vector, and type of transmission. This is a valuable compilation and review on a subject important to veterinary entomologists.

Stable fly.—The stable fly [*Stomoxys calcitrans* (Linnaeus)] is a vicious bloodsucking pest of livestock and is widely distributed. Very little research information has appeared on the biology, habits, and breeding sites of this insect in recent years. Champlain *et al.* (43) and McGregor & Dreiss (44) provide useful new information on rearing the insect in the laboratory since availability of large numbers of flies for insecticide testing and other purposes is important in research.

Effective sprays to protect animals against stable flies have not yet been found that are practical for use on range animals. Pyrethrum and allethrin fortified with synergists are the best materials, but they do not afford protection for more than a few days, when used at maximum practical strengths. Their short and erratic performance precludes general use on range cattle. Moore *et al.* (45) and Bruce & Decker (38, 39) have studied formulations and give results of practical field tests. In research on residual spraying of barns -on various types of farms Dahm & Raun (46) evaluated several organic phosphorus insecticides. Diazinon appeared to be the most effective.

Horn fly.—Before the advent of DDT there was no practical means of controlling the horn fly [*Siphona irritans* (Linnaeus)] on range cattle. Control of this pest on dairy cows or small herds of beef cattle could be accomplished only by daily treatment with mist sprays containing pyrethrum or the organic thiocyanates. It is now our easiest livestock insect to suppress on beef cattle, for sprays of DDT, TDE, toxaphene, or methoxychlor are very effective for three to six weeks. Methoxychlor is the only chlorinated hydrocarbon insecticide recommended for use on dairy animals because the other materials effective for horn-fly control result in excessive residues in the milk. Many experiments have been conducted comparing the effectiveness of different insecticides against the horn fly. State and federal leaflets give recommendations on approved materials and methods of application on both beef and dairy cattle.

Studies on the increase of milk yields when cows were protected against horn flies, stable flies, and horse flies were conducted by Bruce & Decker (47, 38). They showed that herds treated with DDT for protection against horn flies produced significantly more milk than did untreated herds. Herds protected against horse flies with frequent applications of synergized pyrethrum showed a highly significant increase in butterfat production. Laake (48) showed that sprayed cattle in about two months gained about 50 pounds more than unsprayed cattle.

An important contribution in simplifying methods of application of insecticides was the development by Rogoff (49) and Rogoff & Moxon (50) of rubbing devices for horn-fly control. These devices, usually consisting of insecticide-treated burlap sacks wrapped around a chain or cable suspended between two posts, have given excellent control after the cattle learned to rub against them. Further work by Lindquist & Hoffman (51) and others confirmed the usefulness of this method of horn-fly control.

Culicoides.—*Culicoides*, family Heleidae, have become of increasing interest to entomologists and others during the last few years. The taxonomy of the group is being overhauled by several workers. A paper by Foote & Pratt (52) on the *Culicoides* of the eastern United States contains keys to numerous species, with notes on their distribution and biology. Khalaf (53) writing on the speciation of the genus treats the group from a world-wide basis. Other papers by Vargas & Wirth (54), Vargas (55), Kettle & Lawson (56) Forattini (57), Ortiz & Leon (58), Wirth (59), and Williams (60) describe new species or groups of species.

The biology and ecology of the *Culicoides* have not been intensively studied, but recent papers by Blanton *et al.* (61), Woke (62), and Okada (63) present information on several species. Observations on the swarming flight and mating of *Culicoides* are reported by Downes (64), and also information on the biology of two species in Great Britain.

A newly discovered disease of sheep in the United States, at first named "sore muzzle" was reported by Hardy & Price (65). This disease appeared to be similar to one known as "bluetongue" in South Africa. During a visit

to this country R. Alexander, Director of Veterinary Services in the Union of South Africa, and an authority on the disease, confirmed the fact that the American and African bluetongue disease of sheep are similar. *Culicoides* were known to be vectors of the disease in Africa. Price & Hardy (66) isolated the virus from sheep in Texas and performed experiments that suggested *Culicoides variipennis* (Coquillett) was a vector. Their experiments were not conclusive, and positive transmission information will be difficult to obtain until *Culicoides* can be readily reared in the laboratory. Very likely *C. variipennis* is a vector of the agent of bluetongue disease in this country, but other species may also be involved. Research is now under way to determine the distribution and larval habitats, and to develop laboratory methods of rearing, and methods of control of *C. variipennis* and related species.

An allergic dermatitis of the horse was shown by Riek (67) to be caused by hypersensitivity to bites of *Culicoides robertsi* Lee and Reye. This hypersensitivity was associated with an increase in the histamine concentration in the blood during the summer months and reached its maximum during the late afternoon and early evening, when the *Culicoides* were most active. A rise in blood histamine was also observed in susceptible horses for a short period after the injection of an antigen prepared from this fly.

Most of the research on the control of *Culicoides* has been in relation to their attack on man. Treating screens with insecticides and repellents has prevented entry into buildings. Labrecque & Goulding (68) found that granulated dieldrin at 1.25 pounds and BHC at 2 pounds of gamma isomer per acre reduced larval breeding of *Culicoides furens* (Poey) in Florida salt-marshes for 12 to 24 weeks, respectively. Previously sprays applied from the air failed to give satisfactory larval control because the dense cover prevented the penetration of most of the material to the mud flats.

Black flies.—Most species of black flies (Simuliidae) are vicious pests of man and animals, and in addition they are carriers of several disease agents. Taxonomic studies have not been numerous during the last two years, but Bentinck (69) has reported on the black flies of Japan and Korea, giving taxonomic information on the several species as well as notes on the geographical distribution, ecological preferences and biting habits. The Japanese black flies had received relatively little study prior to this publication. The illustrations showing taxonomic characters are excellent. Freeman & de Meillon (70) published a large treatise on the simuliids of the Ethiopian region. A well illustrated treatise on the black flies of New York State with keys to the larvae, pupae, and adult females was presented by Stone & Jamnback (71).

Sommerman *et al.* (72) published a study of the black flies of Alaska, giving special attention to larval habitats and the life histories of approximately 35 species. Only five or six of these black flies are known to feed on humans in Alaska. Sailer (73) gives similar information, stating that surprisingly few biting records have been obtained in several years' observations. Al-

though biting records on man are scarce in Alaska, there is no dearth of information on the vicious biting habits of these flies in other areas. Bierer (74) described severe outbreaks of black flies in the United States when livestock were tortured and even killed by these pests. Curtis (75) reports on cattle-infesting black flies closely related to *Simulium arcticum* Mallock in British Columbia, which cause serious loss of weight in beef cattle by their vicious biting. He also gives some interesting life history information on this species. Edgar (76) reported that an outbreak of *Simulium meridionals* Riley caused egg production of Leghorn hens to drop sharply from 70 to 20 per cent within eight days. After the outbreak the hens recovered and egg production returned to normal.

A comprehensive volume on black flies and their role as transmitters of disease agents in Guatemala by Dalmat (77) presented detailed information on breeding habits, distribution, life history and taxonomy of black flies as well as their relation to transmission of onchocerciasis, an important disease of man in South and Central America and parts of Africa. The book is well illustrated with photographs, maps, and line drawings.

Hocking & Pickering (78) reported on the bionomics of some species of Simuliidae in northern Manitoba. Information is presented on the larval and pupal habits, mating, feeding, oviposition, and parasites and predators of several species. Methods of rearing field-collected larvae in the laboratory are described.

Black-fly breeding in irrigation canals and water drops has been observed by entomologists over a number of years, but little on this subject has been published. Edmunds (79) made a survey in an irrigated area in western Nebraska after farmers had complained of small flies bothering their livestock. He found black-fly pupae and larvae in small numbers along the edges of the main canals, especially where the water was moving swiftly, and in large numbers in the concrete drop structures. Of 206 drop structures examined, black flies were found in 93 per cent of them. Collections were identified as *Simulium vittatum* Zetterstedt and *Simulium griseum* Coquillett. With the enormous increase in irrigation in the Western States it is expected that black flies as well as mosquitoes will increase and cause trouble to livestock growers.

Black flies transmit a leucocytozoon disease of poultry. This disease has been reported from several states but is particularly prevalent in Jasper County, South Carolina. Jones & Richey (80) reported on the biology of black flies and their relationship to the disease. Ecological information was obtained on eight species breeding in the slow-moving, meandering streams but only two of them, *Simulium slossonae* Dyar and Shannon and *Simulium congareenarum* (Dyar and Shannon), were found feeding on turkeys. Reports on the causative parasite and transmission to turkeys have been published by Simms (81), Richey & Ware (82), and Newberne (83).

Control of black-fly larvae has been of considerable interest since Fairchild & Barreda (84) demonstrated that the application of 0.1 to 0.2 p.p.m.

of DDT would rid a stream of larvae for a distance of several miles. This method of control has been tested by many investigators, including Gjullin *et al.* (85), Hocking (86), Vargas (87), and Lea & Dalmat (88), and it proved to be outstanding in reducing the black flies. The effectiveness of controlling black-fly larvae up to several miles by dripping DDT into a stream at low concentrations for periods of 10 to 60 min. is phenomenal.

An experiment in eradication of *Simulium neavei* Roubaud, the vector of onchocerciasis in the Kodera district of Kenya, has been reported by Garnham (89). In 1946 DDT was applied to all streams in the district, and annual surveys since then have failed to yield larvae or adults. Although the incidence of microfilariae in children has been reduced, the disease has not been eradicated. Hocking & Richards (90) and Lea & Dalmat (91) have treated streams in large areas and demonstrated great reductions of black flies. Infiltration of flies from untreated areas hinders their complete elimination.

Although 0.1 to 0.3 p.p.m. of DDT has been effective in various field tests conducted by different workers, low concentrations were not sufficient to kill larvae in laboratory tests. Apparently Lea & Dalmat (92) were the first to report low kill of larvae. They found that exposure to 10 p.p.m. of DDT for 30 min. would not always cause 100 per cent mortality. Apparently the effectiveness in the field is attributable to the fact that DDT causes the larvae to detach from rocks and vegetation, and they are swept downstream. They are then subjected to predation by fish and other animals. Observations such as these emphasize the importance of understanding the biology of an insect and the mode of action of insecticides in evaluating their effectiveness in both laboratory and field tests.

Jamnback & Collins (93) published an informative and well illustrated volume on the control of black flies in New York. Data are presented on the effectiveness against adults of insecticides applied by ground fogging machines, conventional aircraft, and helicopters. Results of tests against larvae and the effects of insecticides on fish, both directly and indirectly, through destruction of their food are given.

Bot Flies

Very little research on the control of the human bot fly, *Dermatobia hominis* (Linnaeus, Jr.), an important pest of cattle in Central and South America, was done prior to 1948. Dips containing arsenious oxide have been used extensively for their control in Central and South America for many years. Laake (94) reported on experiments conducted in 1948 in Brazil with toxaphene sprays on cattle. This insecticide reduced the number of larvae infesting cattle. The grub reduction was probably a result of the destruction of biting flies and mosquitoes that carry the *Dermatobia* eggs which leads to infestation in livestock; however, no data are available to show that the treatment does not affect the hatching larvae.

Neel (95) reported on the use in Costa Rica of sprays containing 0.5

per cent of toxaphene, 0.15 per cent of aldrin, 0.5 per cent of DDT plus 0.03 per cent of gamma BHC. Applications every two weeks for approximately four months significantly reduced the infestations of larvae under the skin. There appeared to be no significant difference between the insecticides used. Adams *et al.* (96) tested 0.5 per cent toxaphene emulsions on Nicaraguan cattle for *Dermatobia* larvae control. The infestations were reduced to about 3 per cent of the controls after six or seven biweekly sprayings.

Garr (97) reported on a new means of controlling the sheep bot fly (*Oestrus ovis* Linnaeus). Of many substances tested, the ether extract of male fern (*Dryopteris filix-mas*) gave best results. The extract was emulsified with an equal amount of alcoholic soap solution in water in which 0.2 per cent of anhydrous sodium carbonate had been dissolved. First-instar larvae dipped in the solution became paralyzed. Infested sheep sprayed in the nostrils with about 10 ml. of the solution soon discharged from one to 200 paralyzed first-instar larvae per animal.

Breev & Karazeeva (98) reporting on *Oedemagena tarandi* (Linnaeus) infesting reindeer in the northwestern part of the Soviet Union, confirmed that the larvae spend 10 months in the body of the host. The warbles usually appear between the fifth and fifteenth of October, and it is important to slaughter the animals before the larvae appear so as to have hides without holes in them. Oviposition and subsequent appearance of grubs under the skin are dependent on temperature. The best time to squeeze out the larvae is in May and early June. Healthy and well-nourished animals were reported least infested.

Experiments to find ways of protecting reindeer from attack by *O. tarandi* by destroying the females during their oviposition period have been reported by Breev & Savel'ev (99). Driving animals through a cloud of finely dispersed DDT or BHC resulted in a sharp decrease of adults and subsequent larval infestations. A method of spraying an entire herd in the open was devised which consisted of attaching spray nozzles to a long pole and drifting the insecticide over the animals. In this way two to three thousand reindeer could be treated in less than two hours.

The development of new sprays for use on the backs of cattle to destroy grubs, *Hypoderma lineatum* (De Villiers) and *bovis* (Linnaeus), has not progressed very fast for several years. Smith & Richards (100) reported preliminary results with several organic phosphorus insecticides applied as washes. Bayer L 13/59, 21/199, and Diazinon at 0.5 per cent destroyed 84 to 92 per cent of *lineatum*, but malathion, EPN, and dieldrin were ineffective at the concentrations used. Similar tests conducted in Oregon by Roth & Eddy (101) indicated that Bayer 21/199 (3-chloro-4-methylumbelliferone-0,0-diethyl thiophosphate) as a wash was effective against *lineatum* and as a spray was more effective than rotenone against *bovis*.

Kühl (102) reports on spraying cattle with DDT and gamma BHC plus chlordane at three-week intervals during the fly season, but neither spray reduced the grub infestations significantly. Raun (103) tested pyrethrum

sprays during the fly season to prevent oviposition by heel flies. An automatic treadle-type sprayer was used to treat the cattle daily. Based on grub-incidence records the spray prevented some oviposition by *lineatum* but not by *bovis*.

An important new approach to possible control of *bovis* by interrupting the larval developmental period in the vertebral canal and tissues was proposed by Lienert & Thorsell (104). The migration of larvae of this species in the host is thought to be a mechanical process or a combination of boring and production of a toxin that dissolves muscle tissue and skin. The authors suggested that enzymes are involved in the forward progression of the larvae and that inhibition of the enzymes might destroy the larvae. Experiments showed that autolysates from larvae collected in the spinal canal exert a splitting effect on the collagens only. Inhibition of the collagen-collagenase system could therefore prevent larvae from developing.

Progress in the control of cattle grubs through the use of systemic insecticides is discussed under *Systemic Insecticides*.

BLOW FLIES

Blow fly strike has been a serious problem to the sheep industry in Australia for many years. Early literature implicated *Phaenicia sericata* (Meigen) as the species chiefly responsible. In the mid-thirties evidence began to accumulate showing that *Phaenicia cuprina* (Wiedeman) was the most important sheep blow fly in Australia and that *P. sericata* was not of economic importance. These two species differed morphologically and in distribution and habits. Waterhouse & Paramonov (105) have described characters whereby larvae and adults of both species may be recognized. They reported on hybridization experiments that indicated there was great difficulty in obtaining successful matings. The authors presented data showing that 58.3 per cent of naturally infected sheep harbored *P. cuprina* and no other species. This species is of little economic importance in the United States, but *P. sericata* oviposits at times on soiled or damp wool and causes losses among sheep.

In Australia *P. cuprina* oviposits on the body of sheep carrying 6 to 12 months' wool and on the crutch. The Mules operation combined with correct docking and tail operations has reduced blow fly strike on the crutch. Waterhouse & Scott (106) found that 2 per cent DDT sprayed on the backs of sheep was the best insecticide tested and in insectary tests protected sheep against oviposition by *P. cuprina* for 6 to 8 weeks. Crude BHC preparations containing 0.5 per cent of the gamma isomer gave valuable protection. Chlordane and toxaphene gave only fair results. In similar studies Riches & O'Sullivan (107) reported that sheep were protected from 6 to 7 weeks with 0.01 per cent gamma BHC, 7 to 8 weeks with 0.01 per cent aldrin and dieldrin, 13 to 14 weeks with 0.25 per cent gamma BHC, and 16 to 18 weeks with 0.25 per cent aldrin and dieldrin. Best results were obtained when the spray was driven into the wool with a straight stream.

LICE

Lice on livestock are important to the stockman, since he must control them in order to insure proper weight gains. Peterson *et al.* (108) found that heavy infestations on cattle produced severe anemia, at times reducing the red-blood cells to such an extent that it was necessary to destroy the lice to prevent the host from dying. When the condition of the animals under study became critical as a result of loss of blood, it was not materially altered by additional feed or improved care.

Satisfactory control of lice on cattle, sheep, goats, and hogs with insecticides has been worked out, and most of the literature on the subject is five or more years old. Readers are referred to Knipling (109) and United States Department of Agriculture leaflets (110, 111) for details of recommended control procedures and insecticides.

In the development of lower cost methods of treatment for control of lice on cattle, the most important advance has been in self-treatment devices. Hoffman (112, 113) found that burlap sacks treated with 5 per cent of a chlorinated hydrocarbon insecticide in oil and wrapped around a post or cable suspended between two posts provided effective control on cattle in feed lots. The effectiveness of such devices depends upon the extent to which cattle rub against them but is less than that obtained by thorough spraying or dipping.

Smith & Richards (114) evaluated several new insecticides against lice on cattle, sheep, and goats when applied as sprays and dips. Strobane and toxaphene at 0.5 per cent concentration were about as effective as DDT against *Haematopinus eurysternus* (Nitzsch) on cattle. Of the organic phosphorus insecticides, malathion at 0.5 per cent, Bayer 21/199 at 0.2 per cent, and Diazinon at 0.1 per cent gave perfect initial kill of lice and prevented reinfestation for two weeks. Parathion at 0.01 per cent and Am. Cyanamid 4124 at 0.25 per cent gave protection for three weeks. These insecticides were also highly effective against *Bovicola caprae* (Gurlt) and *Bovicola limbatus* (Gervais) on goats.

SYSTEMIC INSECTICIDES

One of the important trends in research on control of livestock insects during the last few years has been the increased emphasis on finding insecticides that can be fed or injected into the animals for destroying lice, ticks, cattle grubs, and biting flies. Many investigators have expressed doubt that it would ever be possible to find materials that would act systemically and destroy pests of livestock without seriously damaging the host. The idea does not seem unreasonable, however, because of progress made in control of various infections and parasites by internal medication. The antibiotics, for example, are highly effective against several human and animal diseases. It seems logical to expect success, and progress during recent years confirms the fact that insects feeding externally or internally, such as the cattle grub, can be destroyed by internal medication. However, the problem

of developing an effective and safe material is not easy and entails an immense amount of careful research.

Systemic insecticides for livestock would have value in the control of several kinds of insects, especially cattle grubs. The only effective insecticide now in use is rotenone, which is sprayed on the backs. This method of control has serious limitations. At least two or three thorough sprayings are necessary during the coldest months of the year, and they must be applied when the grubs are in the back after they have already caused considerable damage. A single, effective treatment given when the grubs are still in the body of cattle would provide more practical and very likely less costly control. A similar treatment for the control of lice of cattle, sheep, and goats might also be practical.

Systemic insecticides for control of biting flies probably would not be practical for ordinary use, unless the material remained effective for several days. There are situations, however, where a systemic material of short duration would be useful. For example, in disease outbreaks the treatment of livestock every few days might break the chain of transmission by insects. The problem of finding a safe insecticide that will be retained by the animal and continue to kill bloodsucking insects such as flies for a week or two is indeed formidable, especially when excessive insecticide residues in meat and milk must be resolved.

The idea of internal use of insecticides for control of insect pests of animals is not new. Entomologists and others have considered this type of treatment for 30 years or more. During the 1920's numerous proprietary products flooded the market. These products were purported to control insects and related forms on animals, particularly poultry, when added to the feed. Parman et al. (115) reported work with dozens of chemicals against natural infestations of chicken lice and the fowl tick but concluded that none of them would control external parasites. Emmel (116) and Creighton et al. (117) found internal treatment with sulfur ineffective, although its presence in feed appeared to reduce louse infestations through external contamination of the animal. Although the early workers failed to find promising materials for systemic use, recent research has unearthed leads, and it is expected that practical systemic insecticides will be developed in the future.

Lindquist et al. (118) found that pyrethrum and DDT fed to rabbits in the laboratory had some systemic effect on bed bugs and mosquitoes. De Meillon (119) showed some activity of the gamma isomer of BHC against the bed bug, yellow fever mosquito, and ticks when given to rabbits. Similarly, Garnham (120) showed that the gamma isomer of BHC given orally killed the yellow fever mosquito feeding on the rabbit. Knipling et al. (121), in testing 33 chemicals on rabbits, found some of the indandione compounds effective in killing the human body louse feeding on rabbits, and also confirmed the effectiveness of gamma BHC against mosquitoes. Adkins et al. (122) reported on the testing of 13 chemicals administered orally to rabbits for the control of bed bugs, Cimex lectularius Linnaeus, and the lone-star

tick, *Amblyomma americanum* (Linnaeus). Bayer L 13/59, Bayer 18/178 (0-[2-(ethyl mercapto)methyl]-0,0-dimethyl thiophosphate), and Bayer 21/116 (0-[-2(ethyl mercapto)ethyl]-0,0-dimethyl thiophosphate) caused 100 per cent mortality of bed bugs and ticks feeding on rabbits treated at a rate of 200, 130, and 95 mg./kg. of body weight.

DeToledo & Saur (123) reported that BHC given to cattle showed systemic effect against the human bot fly, *Dermatobia hominis*. Lindquist *et al.* (124) reported that dieldrin, aldrin, lindane, and heptachlor injected subcutaneously in mice caused mortality of the deer fly, *C. discalis*, and *Aedes dorsalis* (Meigen) mosquitoes taking blood meals, but when injected into cattle, they gave poor kills of natural populations of tabanids and mosquitoes feeding on the cattle. Roth & Johnson (125) obtained high kills of grubs in experimental work when dieldrin was injected subcutaneously twice at the rate of 25 mg./kg. of body weight. McGregor & Radeleff (126) and McGregor *et al.* (127) reported good destruction of grubs with dieldrin, aldrin, and lindane. However, these chlorinated hydrocarbon insecticides are not considered satisfactory because large amounts are stored in the fat. Furthermore, the materials did not destroy young grubs in the bodies of the cattle. The same authors also tested organic phosphorus insecticides against the cattle grub. Although some of them showed promise, they did not measure up to the standards of effectiveness and safety required of systemic materials.

Schwartz *et al.* (128) reported on the free-choice ingestion of phenothiazine by cattle on the incidence of *Hypoderma lineatum* de Villers. The evidence suggests that the compound had some effect on larvae in the body of the host. However, observations on cattle herds consuming phenothiazine as routinely used for controlling internal parasites and the results of controlled experiments which are not yet reported in literature cast doubt on the effectiveness of phenothiazine as a grub-control medication.

The papers reviewed on systemic insecticides do not present critical and direct comparative data on their effect on insects in different host animals. Because of differences in assimilation or excretion, or the formation of metabolites of insecticides by different species of animals, there are dangers of overlooking valuable leads or misinterpreting the information obtained. The insects themselves are likely to differ in susceptibility or resistance to any given compound in different hosts. Future research will no doubt provide basic data that will explain apparent inconsistencies in older work and guide the research worker in developing effective systemic insecticides.

SCREW-WORM CONTROL WITH SEXUALLY STERILE MALES

The eradication of the screw-worm, *Callitroga hominivorax* (Coquerel), from the island of Curacao, Netherlands Antilles, by the systematic weekly release of males sexually sterilized by gamma rays, reported by Baumhover *et al.* (129), was one of the most interesting events in recent entomological history. The fact that an insect was completely eliminated from an area, even of only 170 square miles, is of great importance. Of special interest, however, is the unique method of achieving the eradication.

Sterile males were released at the rate of about 400 per square mile per week over a period of several months. The natural production of male screw-worm flies probably equaled 100 to 200 per square mile per week. The sterile males caused a rapid decline in production of flies, which meant that the ratio of sterile to fertile males increased rapidly. The natural screw-worm population decreased enormously in about three months and complete eradication was achieved in five months.

The possibilities of this approach to control of the insect were indicated when Bushland & Hopkins (130, 131) found in laboratory studies that x-rays and gamma rays effectively sterilized the screw-worm. The maximum sterilizing effect with minimum adverse effect on adults was achieved by irradiating pupae. The optimum time was determined to be five to six days after pupation at 80°F. or two or three days before adult emergence. A dose of 2,500 roentgens caused sterility of the males and 5,000 roentgens sterility of the females. These doses of irradiation do not adversely affect sexual behavior. In cage tests under laboratory conditions a given ratio of sterile to fertile males with a population of normal virgin females resulted in a like ratio of sterile to fertile egg masses. The findings of these authors were important milestones in screw-worm sterilization research and led to the field studies described previously.

The gamma-ray source used in the screw-worm experiments was described by Darden et al. (132). This portable unit meets the requirements of maximum irradiation chamber size and amounts of cobalt 60 at reasonable cost without violating accepted radioactive safety standards.

The feasibility of controlling the screw-worm by releasing sexually sterile males was suggested by information obtained over many years by a number of investigators on the life history, ecology, population densities, host relationships, and other aspects of screw-worm biology. Some of the significant observations having a bearing on this problem have been reviewed by Lindquist (133).

Theoretical considerations in the use of the sterilization method for insect control are discussed by Knipling (134). The principle of reducing the population of an insect by sustained release of sterile males among an existing population is a mathematical proposition. If the first release of an insect exceeds the native population by five or ten times, the next generation should be greatly reduced provided the population is stable. Sustained releases in the same numbers originally released will naturally provide for a higher and higher ratio of sterile to fertile insects. In several generations, therefore, the extinction point of the species should be reached. The Curacao experiment bore out the calculated decimation of the screw-worm population to an amazing degree.

On the basis of information available at the present time, the sterilization method will be feasible for relatively few insects. Several definite requirements must be considered in appraising the possibilities of controlling or eradicating an insect. (a) An economical method of rearing millions of insects must be known or capable of development. (b) The insect must be of a

type that can readily be dispersed so that released males can successfully compete with native males for virgin females. (c) The sterilizing method must produce sterility without serious adverse effects on the insect. (d) The females should preferably mate only once, but if multiple matings occur, the irradiated (sterile) males must produce sperm so as to compete with sperm of the native fertile males. (e) The insect to be controlled must have a low population or must be reduced by other methods. The area to be treated must, of course, be reasonably protected against reinfestation. In estimating the possibilities for use of sterilization for control, the above factors should be taken into consideration. Obviously, a thorough knowledge of the habits and ecology of the insect must be available.

RADIOISOTOPES IN RESEARCH

Since 1949 the use of radioisotopes in research on arthropods of medical and veterinary importance has increased greatly. One of the important uses of radioisotopes is in ecological studies. Insects can easily be tagged with these materials and upon later recapture, they can be positively identified with Geiger counters. The tagging procedure can be used in studies on flight, movement or migration in or on the soil, in foods, feeds, and animals. Studies on systemic insecticides in animals can be greatly aided by labeled materials. The uptake of blood, serum, or tissues by insects and determinations of the amount and possible identity of metabolites can be speeded with labeled insecticides. Insect populations in a locality can be determined from ratios of tagged to untagged specimens. The same method can be used to measure the effectiveness of control operations.

Several writers, including Bugher & Taylor (135), Jenkins & Hassett (136), Thurman & Husbands (137), and Provost (138), have reported on experiments with mosquitoes to determine flight range, direction of flight, age, and estimated population densities. In most of these experiments the mosquitoes were tagged with P^{32}. Similar studies on house flies and blow flies have been made by Yates et al. (139), Schoof et al. (140), and Quarterman et al. (141). General review papers on the uses of radioisotopes in insect research include those by Jenkins & Hassett (142), Jenkins (143), and Lindquist (144).

Radioactive insecticides are useful in studies of the absorption, distribution, and degradation of the materials when applied in measured dosages to insects. Several insecticides including DDT, BHC, parathion, pyrethrins, allethrin, and methyl bromide have been labeled with radioisotopes [Dahm (145)]. This author reviews methods of labeling various chemicals and uses which have been made of them.

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TRANSMISSION OF DISEASE AGENTS BY PHLEBOTOMINE SAND FLIES[1]

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Phlebotomine sand flies are known to be carriers of the following diseases: sand-fly fever, the various forms of leishmaniasis, and Carrion's disease. They also cause harara, a skin condition resembling an urticaria, which is a reaction to their bites. Sand flies[2] are probably involved in the transmission of the leishmanias of lizards, but the evidence for this is still incomplete. They are proved carriers of three species of trypanosomes of reptiles and amphibia and have been reported to be suitable hosts for the development of *Wuchereria bancrofti* (Cobbold).

BIONOMICS

The bionomics of sand flies are discussed briefly in so far as they are relevant to epidemiology. The subfamily Phlebotominae has a wide distribution, mainly in the tropics and subtropics. Their northern limit in Europe is Paris, and in Eastern Asia they occur as far north as Manchuria. They are distributed over the whole continent of Africa, South and Central Asia and also occur in the northern parts of Australia and in New Guinea. In America they are widely distributed in South America except its southernmost parts, and in North America some species occur as far north as Washington, D. C. About 150 species have been recorded from the Old World and about 200 from the New World. Sand flies inhabit the most diverse biotopes ranging from arid and semi-arid regions (Sudan, Iraq, Turkestan, N.W. India) to tropical rainforest. They develop in the soil, sometimes at a depth of 20 to 30 cm., in cracks in rock and masonry, in heaps of rubble, wherever suitable temperature and particularly high humidity create favourable conditions. The larvae feed on decomposing organic material, rotting vegetation, faeces of rodents or reptiles, dead insects, etc. The bionomics of relatively few species have been studied, and naturally attention has been focused on species suspected as vectors of agents of human disease. A satisfactory knowledge of the bionomics of any species can be obtained only by field observations carried out over a whole season and by breeding experiments in the laboratory.

In all species investigated so far only the female is haematophagous. There is some evidence, however, that some species also ingest plant juices. This, according to Kirk & Lewis (51), has been shown by Fowler in the Sudan, who

[1] The survey of the literature pertaining to this review was completed in April, 1956.

[2] The term "sand flies" in the present review refers to phlebotomine sand flies only.

by chromatographic analysis demonstrated plant sugars in the contents of the oesophageal diverticula of *Phlebotomus papatasi* (Scopoli). This point is of considerable importance and requires further investigation for, as will be seen later, the capacity of *Phlebotomus argentipes* Annandale & Brunetti to transmit *Leishmania donovani* (Laveran & Mesnil) is enhanced by feeding on raisins.

The alimentary tract of fed and unfed sand flies is bacteriologically sterile. A chance contamination interferes with the digestion of a blood meal and is fatal to the insect. The blood meal is enclosed within a peritrophic membrane, and a new peritrophic membrane is produced with every feed; in species which are liable to refeed while the stomach contains a residue of the previous meal the latter is found enclosed in the shrivelled remains of the old peritrophic membrane [Adler & Theodor (5)]. The peritrophic membrane was redescribed in 1942 by Dolmatova (33).

There are considerable differences in host preference among different species. In the Mediterranean the species of the genus *Sergentomyia* feed mainly on lizards (geckoes) while those of the genus *Phlebotomus* feed on mammals. In tropical Africa some species of *Sergentomyia* [*S. schwetzi* (Adler, Theodor & Parrot) and *S. clydei* (Sinton)] have been observed to feed both on mammals and reptiles. A species may have different feeding habits in different regions, e.g., *Phlebotomus babu* Annandale feeds on geckoes in India and on man in Mauritius. Species which feed on mammals may also show definite host preferences, and this is of considerable epidemiological importance. Thus *Phlebotomus perfiliewi* Parrot in the Plain of Esdraelon in Israel is zoophilic and rarely feeds on man. During the last 30 years only two cases of human visceral leishmaniasis have been found in this area although at one time 20 per cent of the dogs were naturally infected. On the other hand, *Phlebotomus perniciosus* Newstead in Italy and *Phlebotomus major* Annandale in Crete feed readily both on man and dog, and accordingly there is a relatively larger number of human cases although the number of infections in dogs is always greater than in man.

Human serum contains a substance lytic for leishmanias. This factor, one of the gamma globulins, is inactivated after ingestion in some species e.g., *P. papatasi*. In addition to blood, sand flies also ingest cells lying in the dermis outside the blood circulation (e.g., macrophages).

The accessory glands of the oviduct produce a secretion containing numerous granules. In the newly hatched female the glands are nearly empty and no granules are discernible. The granules appear only after the first blood meal [Adler & Theodor (16)]. In dissecting fresh material the presence or absence of these granules should be noted. Females with no or few granules in the glands are newly hatched individuals, while females without a trace of blood in the stomach, with eggs in the early stages of development, but with granules in the accessory glands, are individuals which have digested a blood meal, laid a batch of eggs, and are ready for a refeed. A quantitative

study carried out throughout a season on the absence or presence of these granules should provide valuable information on population dynamics.

Refeeding habits are of decisive importance in studies of transmission and in this respect there are considerable variations among known vectors. While some species (e.g., *P. perniciosus*) will not refeed on blood until they have laid a batch of eggs, others (e.g., *P. papatasi*) may refeed several times without relation to egg laying. Some species can lay a batch of viable eggs after a single feed and can therefore propagate without refeeding. The fact that sand flies refeed in nature after egg laying is established by finding individuals with an empty alimentary tract and granules in the accessory glands of the oviduct and by finding females with one or two ripe eggs remaining in the ovary after the others have been deposited.

Most species of sand flies of the Palaearctic region are subject to a diapause and pass the winter as fourth stage larvae [Adler & Theodor (16); Theodor (92)]. The adults disappear suddenly after the onset of the winter rains in the Mediterranean. Low temperature is the main but not the only factor which induces diapause. The arrest of development in fourth stage larvae is sometimes also found in cultures in summer, but this is probably a result of unfavourable conditions. The hibernating larvae pupate in spring (May to June), nearly all at the same time, and the spring generation of adults thus appears suddenly in great numbers. There is no evidence of a diapause among species in the tropics although they may be less numerous in the colder months.

Humidity is a decisive factor for sand flies and particularly for the gravid female which lays eggs satisfactorily only at a relative humidity approaching 100 per cent. In arid and semi-arid zones where, as in the Sudan, the maximum temperature of the soil surface may reach 76°C. the local species of sand flies escape from lethal atmospheric conditions into a suitable microclimate by living in rodent burrows or fissures in the soil. Young, Richmond & Brendish (104) first demonstrated that *P. papatasi* in Peshawar breeds in fissures in the ground. Subsequently Sergent & Parrot (77) demonstrated rodent burrows as a natural habitat for sand flies. Vlasow (98) and Petrishcheva (71) showed the importance of rodent burrows as habitats of sand flies in Turkestan. Kirk & Lewis (50) in the Sudan have shown the importance of this extensive subterranean environment. In the Sudan, North Africa, and Arabia both species of the genus *Phlebotomus* which feed on mammals and species of the genus *Sergentomyia* which feed mainly on reptiles are found in rodent burrows.

The larvae cannot exist without water in fluid form which, however, may be bound by capillarity. Larvae kept in closed tubes above water, but not in contact with it, will die within 24 hr. They absorb water with their food and through the skin. The larvae of most Psychodidae are aquatic, and the larvae of Phlebotomus may be considered to have originated from aquatic larvae which have become adapted to life in moist soil [Theodor

(94)]. Eggs and larvae of *P. papatasi* can withstand immersion in water for considerable time. Eggs hatch in water, and first stage larvae can survive immersion for 5 days, larvae of the fourth stage up to 14 days, and when removed continue to develop normally. These findings possibly explain the survival of larvae in the soil during the heavy winter rains when their breeding places may be flooded.

These ecological observations elucidate the life history of sand flies in arid zones, and they are of great epidemiological significance since Russian workers have demonstrated that various species of rodents (e.g., *Rhombomys opimus* Lichtenstein) are natural reservoirs of *Leishmania tropica* (Wright) which is propagated among them by sand flies living in their burrows. These findings explain the infections contracted by travellers in areas remote from human habitations. Some species, among them important vectors, are abundant in arid uninhabited zones where they live in rodent burrows and fissures in the soil but are also common in the very different domestic environments of villages and towns (e.g., *P. papatasi*, *Phlebotomus sergenti* Parrot, and *Phlebotomus caucasicus* Marzinowky).

It is probable that sand-fly-transmitted diseases will be progressively eliminated from inhabited localities where modern insecticides are available, but there will still remain transmission under feral conditions. This applies particularly to East Africa and the forest areas of South America. Further information should therefore be obtained on breeding places, animal associations, and seasonal distribution in these areas.

TRANSMISSION OF LEISHMANIASIS BY SAND FLIES

Roger's observation in 1904 on the development of Leishman-Donovan (L.-D.) bodies into leptomonad flagellates and the then known natural occurrence of *Leptomonas* sp. in insects suggested an insect vector of leishmaniasis. The search for a vector resulted in a number of theories including transmission by bed bugs, fleas, mosquitoes, flies, hippoboscids, insects which feed on plant juices containing leptomonads, and even ancylostomes. These theories are today of merely historical interest and were fully reviewed by Wenyon (100).

In the early years of research on transmission of leishmaniasis there were a few suggestions that sand flies may be the vectors, but these were only several among a number of more or less plausible hypotheses and were not investigated experimentally. Pressat (73) and Sergent & Sergent (79) tentatively suggested sand flies as vectors of cutaneous leishmaniasis. Wenyon (99) provided the first real evidence for this hypothesis. He found that 6 per cent of sand flies examined in Aleppo were infected with leptomonads. In 1911 it was impossible to make a specific diagnosis of female sand flies, but in the light of subsequent knowledge on the sand flies of Aleppo [Adler & Theodor (13)], it is safe to assume that Wenyon was working with *P. papatasi* or *P. sergenti*, or both. It is also clear that the infections observed

by Wenyon were acquired by the adult sand fly as a result of feeding on animals or man, for larvae infected with flagellates do not carry the infection to the adult stage [Christophers, Shortt & Barrand (27)]. It is of historic interest that Wenyon fully realized the possible implications of his discovery for he infected himself with *Leishmania tropica* directly from a human lesion and proceeded to Malta, where sand flies were abundant, with the intention of feeding these insects on his lesion. Unfortunately an accident removed the greater part of the lesion, and experimental work on sand fly transmission was delayed for more than a decade. Mackie (55) suggested sand flies as vectors of kala-azar in India.

The following principles should be considered when dealing with problems of *Leishmania* transmission: (a) the proposed vector of any parasitic disease must have a distribution which includes the area of prevalence of the disease and approximately coincides with it; and (b) the proposed vector of leishmaniasis must have a sterile alimentary tract because human leishmanias do not tolerate bacterial contamination.

Sand flies are the only known biting insects which fulfill both these essential conditions. There is no endemic leishmaniasis in the absence of sand flies, just as there is no endemic malaria in the absence of anophelines.

TRANSMISSION OF LEISHMANIA TROPICA

The modern experimental period in this field was initiated by Sergent *et al.* (78). These authors crushed in saline seven female sand flies from a batch caught in Biskra, an endemic focus of cutaneous leishmaniasis, and inoculated them into the arm of a volunteer who subsequently developed Oriental sore. No leptomonad flagellates were found in the material inoculated, but in view of the result obtained it is certain that flagellates were present. The sand flies used in these experiments were diagnosed as *P. papatasi* because no other species of male sand flies were found in the locality in which the females were collected. In 1921 it was still impossible to make a direct specific diagnosis of female sand flies. This was achieved several years later by the introduction of the armatures of pharynx and buccal cavity and of the spermathecae as systematic characters [Adler & Theodor (6)].

Adler & Theodor (7) dissected 3,624 females of *P. papatasi* caught during 1925 in Jericho, an endemic centre of cutaneous leishmaniasis, and found four females naturally infected with leptomonad flagellates. Three volunteers who were inoculated with these flagellates developed lesions of cutaneous leishmaniasis. Cultures were made from the lesions of these experimental infections and were found to be serologically identical with *L. tropica* isolated from natural lesions [Adler & Theodor (8)]. It was thus definitely established that females of *P. papatasi* taken in an endemic area were naturally infected with *L. tropica*. Sand flies were infected by feeding on the lesions of one of the above volunteers; six volunteers were successfully

infected by the inoculation of flagellates from these experimentally infected sand flies, and other sand flies were again infected by feeding on an experimental lesion on one of these volunteers [Adler & Theodor (9)]. Thus a strain of *L. tropica* passed through five successive hosts: (*a*) a naturally infected *P. papatasi*, (*b*) a human volunteer infected from (*a*), (*c*) sand flies infected from (*b*), (*d*) human volunteers infected from (*c*), and (*e*) sand flies infected from (*d*).

The life history of *L. tropica* was studied both in sand flies infected by feeding on human lesions and others infected by injection of Leishmaniform (i.e. L.-D.) bodies from cultures [Adler & Theodor (10)]. Parrot & Donatien (64) independently made similar studies in Algeria. The development of leishmanias in sand flies is briefly as follows: Leishmaniform bodies develop into flagellates and multiply in the stomach of sand flies. Many move forward to the anterior part of the midgut and attach themselves by the tip of the flagellum to the oesophageal valve and to the striated border (rhabdorium) of the epithelium of the anterior part of the midgut (cardia). The forward movement of the flagellates does not depend on the intensity of the infection and occurs in slight as well as in heavy infections. In heavy infections the anterior part of the midgut may appear choked with masses of flagellates. It is, however, not completely blocked, and sand flies with such heavy infections are able to take a blood meal. Through the oesophageal valve flagellates move forward through the oesophagus into pharynx and buccal cavity (cibarium) and in a small unpredictable number of cases into the distal part of the proboscis, between the teeth at the tip of labrum-epipharynx and hypopharynx.

Numerous attempts to transmit *L. tropica* to man by the bite of heavily infected sand flies gave negative results except in one case which, however, was not considered conclusive [Adler & Theodor (12)]. Finally Adler & Ber (4) succeeded in transmitting *L. tropica* by the bite of sand flies which had been infected on a suspension of flagellates in a mixture of 3 parts of 2.7 per cent saline and 1 part of defibrinated blood. Twenty-eight lesions were produced in five volunteers by the bite of 26 sand flies, and 9 sand flies accounted for 18 of these lesions on one volunteer. Flagellates both normal and dead were found in a stained preparation from the exudate of one of the puncture wounds. The negative experiments will be discussed later.

Experiments with other species of sand flies.—Sinton (87) has shown that the distribution of cutaneous leishmaniasis in India coincided with that of *P. sergenti* and that of kala-azar with that of *P. argentipes*. Adler & Theodor (12) infected *P. sergenti* with *L. tropica* by feeding the insects on oriental sores. They showed that the infection rate in *P. sergenti* is higher than in *P. papatasi* fed on the same lesions and proboscis infections are commoner. An interesting fact noted in the development of *L. tropica* in *P. sergenti* is the appearance of numerous "short, thin" flagellates (length 4.7 to 10 μ and flagellum longer than body) [Adler, Theodor & Witenberg (19)]. These may

be the dominant forms in some infections. The significance of this finding is not yet clear. On the basis of inoculations of flagellates from infected sand-flies into humans it was not possible to differentiate between the infectivity of the various types of flagellates found in infected sand flies. Positive results were obtained both with material from sand flies which contained no short forms and with others which did. Human volunteers were successfully inoculated with flagellates from infected *P. sergenti*. *P. major* in Crete was also infected with *L. tropica*. Mills & MacHattie (56) infected *P. papatasi* and *P. sergenti* with a canine strain of *L. tropica* in Baghdad.

Vanni (96) found leptomonads in a wild *Phlebotomus perfiliewi* in Italy; flagellates were present in midgut and pharynx according to the author. His photographs of "pharynx infections," however, show sections through the anterior part of the midgut. In 1939, he (97) designated *P. perfiliewi* as the vector of cutaneous leishmaniasis in the Abbruzzi. He macerated 200 wild sand flies which he considered to be *P. perfiliewi* and inoculated the macerate into a rat. Vanni's experiment would have carried more weight if the sand flies had been dissected individually, specifically diagnosed, and the presence or absence of flagellates noted. There are a number of species domestic in Italy, and it is most unlikely that a batch of 200 wild sand flies should contain one species only, even though *P. perfiliewi* is common in the Abbruzzi. In Sicily where cutaneous leishmaniasis is present, *P. perfiliewi* was not found among the sand flies examined, which were mostly *P. papatasi* and *P. sergenti* [Adler & Theodor (16)].

Animal reservoirs.—It has long been known that in some endemic areas (e.g., Iraq, Iran) dogs are naturally infected with *L. tropica*. Adler & Theodor (14) infected a dog with flagellates from cultures and from sand flies infected experimentally with *L. tropica;* and a human volunteer by inoculation of culture material from a naturally infected dog.

The work of Latischew & Kriukova (53) elucidated the problem of the association of sand flies and animal reservoirs. They found that various gerbils and a ground squirrel were naturally infected with *L. tropica*. *Phlebotomus caucasicus* and *P. papatasi* living in the burrows of these rodents perpetuate the infection among them. *P. papatasi* with natural infections acquired from the rodents infect man as occasion arises. *P. caucasicus* in Turkestan is apparently not domestic, and its role is that of perpetuating the infection among susceptible rodents. *P. caucasicus* in Persia is definitely domestic and feeds readily on man.

SOUTH AMERICAN MUCO-CUTANEOUS LEISHMANIASIS

The prevalence of the disease in man and dog under various climatic and topographic conditions, the numerous clinical forms it assumes (there is still doubt whether *Leishmania brasiliensis* Vienna represents a single homogeneous species), and the large number of species of *Phlebotomus* recorded from South America suggest that a number of vectors are involved.

The search for vectors by South American workers included numerous studies on the distribution of anthropophilic sand flies in endemic foci. Forratini (39) identified 35,753 sand flies from endemic foci in Brazil and found that they belonged to 11 species. He believed that *Phlebotomus intermedius* Lutz & Neiva played an important role in transmission because of its large numbers, its presence in the forest, its tendency to approach human habitations, the readiness with which it fed on man, and the fact that Forratini & Santos (40, 41) had found natural infections with leptomonads in this species. In a study of the distribution of sand-fly species in Sao Paulo they conclude that the results are not easy to interpret. They found the wild rodent, *Cuniculus paca* Linnaeus, with a cutaneous lesion which contained organisms suggestive of Leishmaniform bodies but they are not definite about the diagnosis. The data on distribution suggest that various endemic foci have their particular vectors, but some species suspected as possible vectors have a very wide range, e.g., *Phlebotomus migonei* França in Brazil, Venezuela, and the Argentine. Pifano (72) in Venezuela found flagellates (5μ long) in saline suspensions of *Phlebotomus longipalpis* Lutz and Neiva *P. migonei*, and *P. intermedius*. Biagi (22) maintains that *P. cruciatus* Coquillett is the only anthropophilic sand fly with a distribution corresponding to that of the disease in Escárcega in Mexico. In Brazil, *P. intermedius*, *P. fischeri* Pinto, *P. pessoai* Coutinho, *P. migonei*, and *P. whitmani* Antunes & Coutinho have been implicated, but these species have not been recorded from endemic foci of the disease in Peru. Pessoa & Coutinho (70) found natural infections of sand flies in endemic foci in Brazil in the following species: 6 out of 2,832 in *P. migonei*, 10 out of 4,154 in *P. whitmani*, and 5 out of 2,267 in *P. pessoai*. The localisation of the flagellates is not described, but Coutinho (28) recorded a natural infection in *P. pessoai* with invasion of the pharynx.

Aragão (20) infected a dog by the inoculation of five crushed *P. intermedius* three days after the sand flies had fed on a lesion. *P. papatasi* was infected by feeding on cultures of *L. brasiliensis* [Adler & Theodor (10)]. The flagellates remained confined to the stomach and did not ascend to the oesophageal valve. When the experiment was repeated later with a recently isolated strain infections of the oesophageal valve were found. Pessoa & Coutinho (70) fed *P. fischeri* and *P. whitmani* on experimental lesions of a monkey. One *P. fischeri* out of 246 and one *P. whitmani* out of 46 became infected. The localisation of the flagellates in the infected sand flies was not mentioned. The disease is acquired both in settled communities and in uninhabited regions, particularly in forests, and it is therefore most likely that in addition to the domestic dog other animal reservoirs play a role in the propagation of *L. brasiliensis*.

The published records contain very little information on the behaviour of the flagellates in South American sand flies, and there is as yet insufficient experimental evidence on the transmission of *L. brasiliensis* by specific

vectors. While it can be expected that the behavior of strains of *L. brasiliensis* in their vectors will conform to that of the Old World leishmanias, more detailed experimental evidence is needed.

VISCERAL LEISHMANIASIS

The three distinct epidemiological types of the disease have to be considered, for the epidemiology is determined by the bionomics of the vector and the role of an animal reservoir.

In India the disease occurs in endemic and epidemic form, no animal reservoir is known, and the epidemiology can be explained without recourse to a hypothetical animal reservoir. In Indian kala-azar all age groups are involved, but mostly young adults.

In the Mediterranean, the Caucasus, Turkestan, China, and South America, regions which differ considerably in their sand-fly fauna, dogs, jackals, and "foxes" (*Lycalopex*) serve as a reservoir from which sand flies are infected and transmit the disease to man. In this type of endemic area, the epidemiological picture is fairly constant in stable populations, and characteristically the number of cases in dogs by far exceeds that in man (e.g., in Malta where the infection rate in dogs was 11 to 13 per cent, the number of human cases was about 90 per annum in a population of 250,000). In the Mediterranean over 80 per cent of all cases are children up to the age of five.

Kirk (48) has shown that the epidemiology of visceral leishmaniasis in the Sudan differs considerably from that in other areas. Cases are sporadic, but the disease may assume epidemic proportions in which event the distribution of cases is not uniform. The disease can be acquired during transit through uninhabited regions, infections in dogs are unknown, and no animal reservoir has been found so far, although Kirk (49) has given cogent reasons for suspecting one. It is in this type of endemic area that the bionomics of feral sand flies are of fundamental importance. The work of Heisch (42) indicates that Kenya resembles the Sudan in this respect.

The transmission of Indian kala-azar by Phlebotomus argentipes.—Knowledge of the transmission of Indian kala-azar is based entirely on the work of the kala-azar commission of India and the kala-azar ancillary enquiry. After many fruitless attempts by many investigators to discover the vector, Knowles, Napier & Smith (52) found that *P. argentipes* became infected with leptomonad flagellates after feeding on cases of kala-azar. Christophers, Shortt & Barraud (26) again infected *P. argentipes* by feeding on human cases and noted that flagellates became attached to the epithelium of the midgut, that the infection may be heavy on the fifth day, and they further observed the invasion of the pharynx by flagellates. Transmission by bite was finally proven 18 years after *P. argentipes* had been incriminated experimentally as vector. This fundamental advance was possible only as a result of basic research on the bionomics of *P. argentipes*. In the following only the salient steps of this work are reviewed.

Shortt, Barraud & Craighead (81) recorded a massive infection of the pharynx and buccal cavity of *P. argentipes*. The same authors (82) found a wild *P. argentipes* naturally infected with flagellates indistinguishable from those found in experimental infections. Later they found seven infections in 326 sand flies caught in kala-azar houses.

No further progress was made till the same authors devised a method of keeping sand flies alive and inducing them to refeed after oviposition. Refeeding sand flies in the laboratory, except in the case of *P. papatasi*, constitutes a problem in all experiments on sand fly transmission which has to be solved for each species separately. In the case of *P. argentipes* keeping the sand flies at a constant temperature of 27°C. proved the most important factor after all the other conditions had been satisfied.

Subsequently a large number of attempts were made to infect human volunteers and the highly sensitive Chinese hamster (*Cricetulus barabensis griseus* Milne-Edwards) by the bite of infected sand flies. Shortt *et al.* (84) reported isolated transmissions to Chinese hamsters, but the large number of negative experiments was impressive and required explanation. Shortt, Craighead & Swaminath (83) failed to infect volunteers by allowing 1,247 infected *P. argentipes* to feed on them. This experiment was carried out during an epidemic of kala-azar when large numbers of human beings became naturally infected. Napier (58) recorded a negative result after feeding 458 *P. argentipes*, previously fed on a case of kala-azar, on a volunteer.

Smith, Halder & Ahmed (88) introduced a variation in their experiments in allowing infected sand flies which had survived oviposition to feed on raisins before refeeding on an experimental animal. They succeeded in infecting golden hamsters (*Mesocricetus auratus auratus* Waterhouse) without difficulty. Using the same method, Swaminath, Shortt & Anderson (90) transmitted the disease to five out of six volunteers. Thus the problem of transmission of Indian kala-azar, which had engaged workers for almost 40 years, was solved. The final decisive experiment raised the important problem of the effect of plant juices on the capacity of *P. agentipes* to transmit *Leishmania donovani*.

The workers in India found infection rates up to 40 per cent in *P. argentipes* fed on human cases (including cases of dermal leishmanoid), a finding of great epidemiological significance and in sharp contrast to the relatively low infection rates obtained by feeding sand flies on human cases in China and in the Mediterranean. In India man serves as an efficient reservoir from which sand flies are infected and transmit the disease to other men.

Transmission of visceral leishmaniasis and animal reservoirs in China.— Young & Hertig (102) infected *Phlebotomus chinensis* Newstead by feeding them on infected Chinese hamsters and on suspensions of L.D. bodies obtained from Chinese hamsters. The infection rate in these sand flies was high (195 out of 384), while that of *Phlebotomus mongolensis* Sinton fed on infected Chinese hamsters was relatively low (16 out of 661). As in the

case of *P. argentipes* infected with Indian *Leishmania donovani* the flagellates assumed an anterior position. *P. chinensis* fed on human cases failed to become infected. Patton & Hindle (68) fed *P. chinensis* on infected Chinese hamsters, and 122 out of 157 became infected. In five sand flies flagellates were found in the pharynx and in two of these the infection extended to the anterior part of the buccal cavity. These observers pointed out that sand flies which had refed showed very heavy infections and that the flagellates were therefore not adversely affected by the ingestion of fresh blood.

Sun and co-workers (89) found 7 out of 421 *P. chinensis* collected in kala-azar houses infected with flagellates indistinguishable from those seen in experimentally infected sand flies. These sand flies probably were infected by feeding on a naturally infected dog as the infection rate on humans is very low. Only 5 out of 102 *P. chinensis* and none out of 202 *P. mongolensis* fed on untreated human cases became infected [Hindle (46)]. Flagellates were found in 430 out of 1170 *P. mongolensis* fed on Chinese hamsters, but these were mainly confined to the posterior part of the midgut. In no case were flagellates found in the oesophagus or pharynx.

The low infection rate produced by feeding *P. chinensis* on human cases indicated that man was not the main reservoir from which the disease was disseminated and that an animal reservoir was involved. Young & Hertig (103) had looked for an animal reservoir in rodents (particularly in view of the extreme susceptibility of the Chinese hamster, a common rodent in endemic areas) and had found no natural infections.

Lee (54) and Feng, Chung & Hoeppli (37) recorded canine visceral leishmaniasis in Peking. Feng & Chung (35) further found that *P. chinensis* fed on naturally infected dogs showed a high infection rate and heavy infections. These findings cleared up the epidemiology of the disease in China.

Transmission of visceral leishmaniasis and animal reservoirs in the Mediterranean.—Three species of sand flies, *Phlebotomus perniciosus, P. major,* and *P. longicuspis* Nitzulescu have been shown to be involved in the transmission of visceral leishmaniasis in this region. Parrot, Donatien & Lestoquard (66) infected four out of five *P. perniciosus* on a naturally infected dog. Adler & Theodor (15) fed both wild and laboratory bred *P. perniciosus* and *P. papatasi* on an infected Chinese hamster. Fifteen out of 18 *P. perniciosus* became infected, but only one out of 123 *P. papatasi.* During 1930 in a large number of feeding experiments on Chinese hamsters the infection rates were 96.8 per cent in *P. perniciosus,* 12 out of 13 in *P. major,* 3.6 per cent in *P. papatasi,* and nil in *P. sergenti.* The comparative susceptibility of *P. perniciosus* and *P. papatasi* to *Leishmania infantum* Nicolle can be appreciated from the fact that on one Chinese hamster only one out of 134 *P. papatasi* became infected at a time when 83 per cent of *P. perniciosus* became infected on this animal, though *P. papatasi* takes a bigger meal and presumably ingests more parasites than *P. perniciosus* [Adler & Theodor (16)].

In Malta Adler & Theodor (17) infected *P. perniciosus* by feeding them on naturally infected dogs. The infection rate varied with the intensity of the skin infection. In dogs the whole unbroken skin is infected with *L. infantum;* parasites are scarce in the blood and sand flies become infected by ingesting infected macrophages from the dermis. On one animal 100 per cent of sand flies became infected, on another 17 out of 25, and on a third, 11 out of 34. *P. major* from Sicily was found to be even more susceptible than *P. perniciosis* to *L. infantum*. On one dog, 31 out of 119 *P. perniciosus* (25 per cent) and 25 out of 31 *P. major* (80 per cent) became infected [Adler & Theodor (18)]. The infection rate increases progressively with the infection in the skin and may attain 90 per cent or more. The susceptibility of *P. perniciosus* to *L. infantum* is such that 20 per cent of the sand flies may become infected on dogs in which the skin infection is too slight to be detected by histological examination.

As in the case of Chinese kala-azar the infection rate obtained by feeding sand flies on very severe human cases was low (4 out of 65 *P. perniciosus*) [Adler & Theodor (16)]. This indicates that man plays a minor role in the dissemination of the disease in this area. It was further shown that in a number of *P. perniciosus* infected on Chinese hamsters flagellates invade the mouth parts and can be recovered from them by placing the sand fly in an apparatus devised by Hertig & Hertig (45). In one experiment a negative result was obtained with a sand fly which had an infection in pharynx and buccal cavity. In sections of a sand fly infected on a Chinese hamster by the authors, Wenyon (100) noted an infection of the distal part of the proboscis between the teeth of the labrum epipharynx and the tip of the mandibles (see Fig. 1).

As in the case of *L. tropica* it was not found possible to associate specific morphological types of flagellates with infectivity for the vertebrate host. A number of sand flies were found to contain almost a pure infection of "short forms," and these were infective for hamsters but infections were also obtained with material from sand flies in which these forms were not observed.

Parrot & Donatien (65) showed that *Phlebotomus longicuspis* is also a probable vector of *L. infantum* in Algeria. Forty-one females of *P. longicuspis* of a batch collected from kennels housing infected dogs were found to contain flagellates.

The epidemiological picture in Turkestan is similar to that in Mediterranean countries but is complicated by the presence of both *L. infantum* and *L. tropica* in man and dog. Chodukin (25) demonstrated skin lesions containing leishmanias in dogs infected with visceral leishmaniasis. *P. major* has been indicated as the local vector. Apart from dogs, jackals have been found naturally infected.

Transmission of visceral leishmaniasis and animal reservoirs in South America.—Following Penna's (69) discovery of 41 cases of visceral leishmaniasis during 47,000 biopsies for yellow fever in Brazil, it was shown that
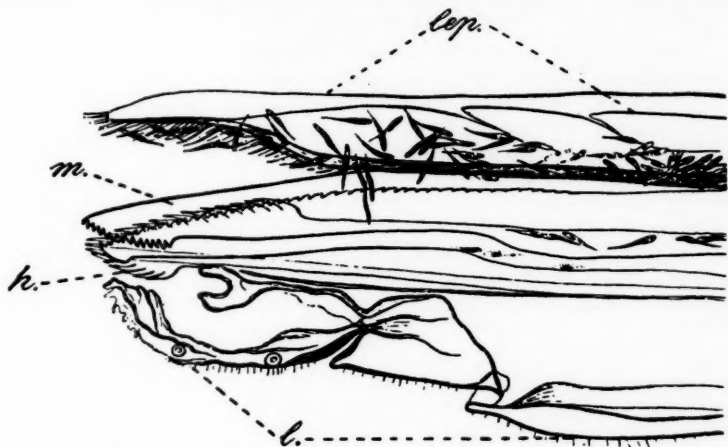
F<small>IG</small>. 1. Flagellates of *Leishmania infantum* at tip of proboscis of *Phlebotomus perniciosus*. lep. = labrum epipharynx; m. = mandible; h. = hypopharynx; l. = labium. ×750. (Drawing of preparation made by Adler & Theodor in Catania.) [After Wenyon (100, p. 319).]

the disease was widely distributed throughout South America, and cases have been recorded from the Argentine, Paraguay, and Colombia, but the majority of cases have been recorded in Brazil. Ferreira, Deane & Mangabeira (38) during investigations in Aleacte found an infection rate of 1.48 per cent in humans, of 4.49 per cent in dogs, and one cat out of 38 was found infected. Deane & Deane (30) working in Ceara reported natural infections in domestic dogs (39 out of 375) and in the wild dog, *Lycalopex vetulus* (Lund) (4 out of 33), in which the disease resembles that of the domestic dog. Recent work of Deane & Deane (31) has thrown a new light on the subject. They found natural infections in *Phlebotomus longipalpis* and produced experimental infections in this sand fly by feeding them on infected humans, dogs, and *L. vetulus*. Four out of 14 human cases were infective for the sand flies, and an infection rate of 14.8 per cent was produced in them by feeding on these cases. Fifty-nine out of 238 sand flies fed on dogs became infected. Ten sand flies fed on *L. vetulus*, and all became infected. In some of these ten sand flies the flagellates invaded the oesophagus, and in one case "small, thin" flagellates were observed in the distal portion of the epipharynx. On the basis of these experiments the authors concluded that *L. vetulus* is the most important natural reservoir of the disease. The most striking point in the findings of Deane & Deane is the relatively high infection rate in *P. longipalpis* fed on human cases. This infection rate is higher than any yet recorded in the Mediterranean region.

DISCUSSION

The mechanism of transmission and the problem of vector specificity are two fundamental points which remain to be discussed.

Mechanism.—Both in the case of *L. tropica* and *L. donovani* there were a large number of experiments in which heavily infected sand flies fed on man and susceptible animals with negative results. At least some of these experiments involved sand flies with an infection of the pharynx and buccal cavity. It is therefore reasonable to conclude that intensity of infection and invasion of pharynx and buccal cavity in themselves are not sufficient to ensure transmission by bite. In an experiment in which a heavily infected *P. perniciosus* was placed in a Hertig apparatus, no flagellates were found in the capillary tube although pharynx and buccal cavity were subsequently found to contain flagellates. Loss of infectivity for mammalian hosts cannot account for the numerous negative results, for when the flagellates of these sand flies were injected into man in the case of *L. tropica* and into Chinese hamsters in the case of *L. donovani*, they were found to be infective. The only possible explanation for the negative experiments is that the flagellates did not enter the puncture wound, i.e., they did not descend sufficiently distally in the proboscis. Positive results were finally obtained by slight but decisive changes in experimental technique, in the case of *P. argentipes* by allowing the infected sand flies to feed on raisins after oviposition and before the refeed and in the case of *P. papatasi* by adding saline to the infecting material. These changes facilitated the descent of the flagellates to the tip of the proboscis. The descent of the flagellates thus depends on factors which influence the behaviour of the flagellates in the proboscis, and an infection of the buccal cavity per se does not ensure this descent in spite of the absence of mechanical obstruction. The factors involved apparently differ in different sand flies. The *P. papatasi* which transmitted *L. tropica* by bite were laboratory bred and had no access to plant juices. Napier (59) has introduced the conception of the "blocked" sand fly. He considers that a heavy infection in the buccal cavity obstructs the ingestion of blood and that this obstruction is overcome during the act of biting by regurgitation and the ejection of flagellates into the puncture wound. We do not think that this hypothesis is justified. The powerful dilator muscles of buccal cavity and pharynx and the elasticity of the epithelium of the cardiac valve are in our opinion capable of widening these organs sufficiently to allow the passage of blood into the stomach [Adler & Theodor (16)]. The analogy with the "blocked flea" is thus not justified.

There is no mechanism for the ejection of flagellates as they do not invade the salivary glands. We therefore think that flagellates are simply deposited from the proboscis into the puncture wound if the distal part of the proboscis is infected. That no activity of the flagellates is necessary appears from the fact that both active and inactive flagellates were recovered from an infected *P. perniciosus* in a Hertig apparatus and from a puncture wound in

human skin immediately after the bite by a sand fly infected with *L. tropica* [Adler & Ber (4); Adler & Theodor (16)].

Vector specificity.—Vector specificity in sand flies, apart from host preference which has been discussed previously, is manifested by a high infection rate following the ingestion of relatively few parasites and is associated with a definite pattern of behaviour of the flagellates which in the case of mammalian leishmanias assume an anterior position in the sand fly. The vectors of the visceral leishmanias of man and dog in the Old World are confined to sand flies of the Major group (Subgenera *Adlerius, Larroussius,* and *Euphlebotomus*). The vectors of *L. tropica* are *P. papatasi, P. sergenti,* and the closely related *P. caucasicus.* These three species are not equal in their capacity to transmit. *P. sergenti* is a more efficient vector of *L. tropica* because its infection rates are higher and proboscis infections are more common than in the other two species.

There are differences among geographical strains of *Leishmania* in their infectivity for the same species of sand fly, and it is therefore not always safe to apply the results of investigations in one endemic focus to another. It was shown experimentally that Cretan strains of *L. tropica,* in contrast to Jericho strains, produce low infection rates in *P. papatasi* both from Crete and Jerusalem [Adler, Theodor & Witenberg (19)].

This specificity is not rigid as in the case of malaria, *e.g., P. major* in Crete was infected with *L. tropica* by feeding on human lesions. *P. major* is not a carrier of *L. tropica* under natural conditions as indicated by the fact that its distribution does not correspond with that of cutaneous leishmaniasis.

The intrinsic factors which determine vector specificity are not known, but they can be influenced quantitatively by feeding sand flies on large numbers of parasites such as the insects are not likely to encounter in nature. Sand flies can be infected with alien leishmanias by feeding them on rich suspensions of parasites, e.g., Adler (3) infected 130 out of 140 *P. papatasi* by feeding them on a suspension of circ. 500,000 L.D. bodies per 0.1 c.mm. of a Sudan strain of *L. donovani* although this sand fly does not transmit *L. donovani* under natural conditions. In many of the infected sand flies the flagellates adopted an anterior position, but only 2 out of 51 *P. papatasi* fed on a hamster heavily infected with the same strain became infected, and in both sand flies the flagellates were confined to the stomach. In this type of experiment the infection rate produced in a nonvector depends on the number of parasites per infecting feed. This explains the occasional infections produced in the laboratory by feeding nonvectors on heavily infected hamsters e.g., *P. mongolensis* on *L. donovani* in China and *P. papatasi* on *L. infantum,* but in these cases the infection rate is always very much lower than in the normal vector. It appears that there is little or no resistance to a *Leishmania* in specific vectors, whereas in nonvectors there is a considerable resistance which can be broken down by large doses of parasites. Once this resistance to an alien *Leishmania* has been broken down, there is a tendency

for the infection rate to decrease with time, i.e., flagellates are destroyed in the stomach of the sand fly, but this too is influenced by the number of parasites in the infecting feed. In the above mentioned experiment with Sudanese *L. donovani* the infection persisted for 15 days.

The infection can also apparently be influenced by the composition of the infecting feed; high infection rates with the Cretan strain of *L. tropica* were produced in *P. papatasi* by reducing the amount of serum in the infecting feed to 10 per cent [Adler (1)]. The course of digestion of a blood meal also influences the behaviour of a *Leishmania* in its vector. Adler, Theodor & Witenberg (19) found that flagellates in the stomach of *P. major* from Crete infected with *L. infantum* did not progress anteriorly when haemoglobin crystals were present, whereas in individuals which had digested a blood meal normally the flagellates progressed to the oesophageal valve and beyond.

The hygienist is naturally interested in the problem of whether a strain of *Leishmania* can establish itself in a new area and create a new endemic focus. It is impossible to answer this question on the basis of the inadequate experimental evidence available. Hindle (47) fed *P. chinensis* on a hamster infected with an Indian strain of *L. donovani*. Sand flies became infected, but the flagellates did not attach themselves to the epithelium of the midgut in contrast to Chinese *L. donovani*. Hindle's experiments indicate that *P. chinensis* is not an efficient vector of Indian *L. donovani*. The following species of sand flies which do not occur in India have been infected with an Indian strain of *L. donovani* by feeding them on a hamster: *P. perfiliewi*, *P. major syriacus* Adler & Theodor, *P. chinensis simici* Nitzulescu [Adler & Theodor (18)].

The clinical resemblance between Old and New World visceral leishmaniasis of man and dog raises the question whether the disease was imported by infected dogs from the Mediterranean during the Spanish conquest or whether, like muco-cutaneous leishmaniasis, it is indigenous in America. Visceral leishmaniasis in the Mediterranean is remarkably stable in its epidemiology and is not known to have established itself in new foci in recent times. Transmission is by sand flies of the Major group only, and *L. infantum* has not adapted itself to other sand flies in spite of ample opportunity. Infection rates as high as 15 per cent, such as Deane & Deane have obtained in *P. longipalpis* on human cases, are not known from Mediterranean endemic centres where canine and visceral leishmanias occur together. The sand flies of the New World are systematically very different from those of the Major group, and it would be strange if a *Leishmania* rigidly restricted to the Major group would adapt itself to American sand flies. For this reason Adler (2) suggested that American visceral leishmaniasis, like the muco-cutaneous disease, is indigenous; but the problem remains to be investigated experimentally by feeding American sand flies on dogs or hamsters infected with Mediterranean strains of *L. infantum*.

Haemoflagellates of lizards transmitted by sand flies.—Leishmanias of

lizards, though quite distinct, have group agglutinins in common with mammalian leishmanias which they resemble morphologically. A study of their biology and transmission is therefore of considerable interest. David (29) infected *P. papatasi* by feeding them on cultures of *Leishmania agamae* David, a parasite of *Agama stellio* Linnaeus. Heavy infections were produced, and in contrast to the human leishmanias the flagellates did not progress anteriorly but descended to the hindgut. *P. papatasi* could not be induced to feed on *A. stellio*. Adler & Theodor (11) infected *P. papatasi* by feeding on cultures of *Leishmania ceramodactyli* Adler & Theodor. The infection rate was high even in experiments in which relatively few flagellates were ingested, and the parasites descended to the hindgut. In Malta *Sergentomyia minuta* (Rondani) was infected by feeding on *Tarentola mauritanica* Linnaeus [Adler & Theodor (18)]. The flagellates were observed only in three sand flies in which heavy infections were seen, and the oesophageal valve was invaded. Parrot (63), on the other hand, recorded a posterior position of flagellates in *Sergentomyia minuta parroti* Adler & Theodor and *Sergentomyia antennata* (Newstead) fed on *T. mauritanica* in Algeria. Heisch (42) records natural infections with leishmanias in anterior position in *S. clydei*. The sand fly had probably become infected by feeding on a lizard.

The flagellates of lizard leishmaniasis adopt an anterior or posterior position according to the species. Shoshina (86) found flagellates in the midgut and hindgut of *Sergentomyia dentata* Sinton ($ = P.\ minutus\ arpaklensis$) from an endemic area of cutaneous leishmaniasis and concluded that *L. tropica* can be transmitted by contamination as well as by bite. It seems probable, however, that Shoshina was dealing with the insect stages of a lizard *Leishmania*.

Three species of trypanosomes are known to be transmitted by sand flies: *Trypanosoma platydactyli* Catouillard by *Sergentomyia minuta* in Malta with trypanosomes in the anterior position [Adler & Theodor (18)]; *Trypanosoma phlebotomi* Mackie by *Sergentomyia babu shorttii* Adler & Theodor in India [Shortt & Swaminath (85)]; and *Trypanosoma bocagei* França, a parasite of the toad *Bufo bufo gargarizans* (Cantor) by *Sergentomyia squamirostris* Newstead in China [Feng & Chung (36)].

The various leishmanias and trypanosomes transmitted by sand flies show stages in the evolution of transmission from the posterior to the anterior position, i.e., from transmission by contamination to transmission by bite.

CARRION'S DISEASE (OROYA FEVER, VERRUGA PERUANA)

Townsend (95) was the first to put forward the theory of sand fly transmission of the disease on the grounds of the close correspondence between the distribution of the disease and *Phlebotomus verrucarum* Townsend, the only species he found in endemic centres. Subsequent research has fully confirmed this theory. The demonstration of Noguchi & Battistini (61) that *Bartonella bacilliformis* (Strong *et al.*) can be readily cultivated, facilitated the

evaluation of transmission experiments and the detection of symptomless carriers.

Shannon (80) found three species, *P. verrucarum*, *P. noguchii* Shannon, and *P. peruensis* Shannon in an endemic centre in Peru. Nogochi *et al.* (62) transmitted the disease to monkeys in New York by the intradermal inoculation of suspensions of wild sand flies transported from an epidemic centre in Peru. Four positive results were obtained from ten batches, totalling about 290 females. It was not possible to distinguish between the females of *P. verrucarum* and *P. noguchii*, and it was thought that the positive results were attributable mainly to the inoculation of *P. noguchii* because males of this species were predominant in the material collected. Battistini (21) succeeded in cultivating *Bartonella bacilliformis* from suspensions of wild sand flies in one out of five attempts involving 200 sand flies. He transmitted the disease to a monkey by keeping it in a screened cage with 30 wild sand flies and infected another monkey by the injection of a suspension made from 30 wild sand flies. Hertig (44) examined specimens from the batches which provided material for Battistini's experiments and found among them two species, *P. noguchii* and *P. verrucarum*.

The above experiments confirmed Townsend's theory of transmission by sand flies and showed that there is a relatively high infection rate in wild sand flies in an endemic focus, but the problem of the mechanism of transmission still remained to be solved, and it was not clear whether one or more vectors were involved. These two points were elucidated by Hertig (44). In his transmission experiments with wild sand flies precautions were taken against possibilities of infection other than by the bites of sand flies. *P. noguchii* was excluded as a possible vector because Hertig found that this sand fly does not feed on man and large animals and restricts its feeding to field-mice. *P. peruensis* was also excluded on epidemiological grounds and since its presence in batches of sand flies used for transmission experiments would have been readily detected because of its large size and other characters. Five out of eight monkeys were infected by the bite of wild sand flies. Hertig's experiments also showed a relatively high infection rate in wild sand flies caught in an endemic centre (at least one out of 55 sand flies known to have fed). It thus became evident that *P. verrucarum* is the only proved carrier of the disease in Peru.

Hertig (44) found proboscis infections with rod-like and coccoid microorganisms in wild *P. verrucarum*. Two species of microorganisms were present in these proboscis infections, and one of them was definitely proved to be *B. bacilliformis*. Cultures of *B. bacilliformis* were obtained from the proboscis of 2 out of 300 sand flies examined, and the identity of the organism was fully established by inoculation into monkeys. The organism was seen in fresh preparations on and between the teeth of the epipharynx. Hertig, with extreme caution, did not claim that these findings definitely elucidated the mechanism of transmission, but it is obvious that microorganisms, even non-

motile, present in the distal part of the labrum-epipharynx of sand flies are inevitably deposited in the skin during the act of biting as in the case of *L. tropica* in *P. papatasi* (See p. 208).

Carrion's disease was considered to be restricted to a number of endemic foci in Peru, but Patiño Camargo (67) reported an outbreak in Colombia, and Hertig (43) cultured *B. bacilliformis* from a case in Ecuador. Brumpt & Brumpt (24) investigated the disease in Colombia and collected sand flies from the infected area. Ristorcelli & Dao Van Ty (74) examined the collection of sand flies from Colombia and stated that it contained no species recorded from Peru. They described two new species, *Phlebotomus osornoi* and *P. columbianus* and a new variety, *P. monticolus var. incarum*. Rozeboom (75) examined sand flies collected in infected areas in Colombia. He concluded that *P. columbianus* is very closely related to *P. verrucarum*. There seem to be no definite characters by which the females of these two species can be separated. Slight differences were found in the male genitalia. *P. columbianus* was the predominant species in the material obtained by Rozeboom from the endemic zone in Colombia. It is therefore reasonable to assume that a species very close to *P. verrucarum*, if not identical with it, transmits the disease in Colombia.

In the case of Carrion's disease epidemiology and experimental evidence both reveal a rigid vector specificity, so far confined to one or possibly two closely related species.

PHLEBOTOMUS (PAPATACI OR SAND-FLY) FEVER

There are no definite diagnostic criteria by which sand-fly fever can be distinguished from clinically similar virus diseases. In endemic centres diagnosis of sand-fly fever is justified only when cases occur in relatively large numbers during the sand-fly season. There is no doubt that epidemics caused by other insect borne viruses have been confused with sand-fly fever in the past. Thus many cases of a recent epidemic of West Nile fever in Israel would formerly have been diagnosed as sand-fly fever on clinical grounds.

The disease is considered to be prevalent in the Mediterranean between April and October [(Sabin, Philip & Paul (76)], but an epidemic in Mediterranean countries (except near the Dead Sea) during April could not possibly be sand-fly fever because *P. papatasi* is still hibernating.

Transmission by the bite of *P. papatasi* was proved by Doerr, Franz & Taussig (32) who infected volunteers by the bites of wild sand flies, some of which had previously fed on cases of the disease during the febrile stage. Doerr's findings have been fully confirmed by other workers who used laboratory bred sand flies for transmission experiments [Whittingham (101); Moshkowsky & Demina (57); Sabin, Philip & Paul (76)].

The epidemiological evidence points to passage of the virus through the egg of *P. papatasi*. Adults of this species disappear in November in the

Mediterranean with the onset of the winter rains and do not reappear till May, an interval more than sufficient to ensure the disappearance of the virus from the blood of human cases. The first cases of the disease appear in May at the beginning of the sand-fly season, and it therefore appears that the virus persists in the hibernating larvae throughout the winter months. Whittingham (101) transmitted the disease to volunteers by the bites of sand flies bred in the laboratory in England, where *P. papatasi* does not occur. The sand flies used in this experiment had no opportunity of acquiring the virus from human cases. He suggested that the transmitting sand flies had acquired the infection by feeding on the corpses and excreta of their parents. Moshkowsky & Demina (57) infected volunteers by the bites of laboratory bred sand flies raised from the eggs of infected females. Sabin, Philip & Paul (76), on the other hand, failed to transmit the infection either by the bites of sand flies which had been allowed to feed on lyophilised infected human serum during the larval stage, or by the bite of laboratory bred sand flies, the immediate offspring of females which had been proved infective. As previously stated, however, in spite of these negative results, the epidemiological evidence points to transovarian transmission in *P. papatasi.*

Sabin, Philip & Paul (76) obtained their negative results working with active larvae. There is a possibility (which has not yet been investigated) that hibernating larvae are a more suitable medium for the survival of the virus than active larvae from which they differ physiologically.

Newstead (60) suggested *P. perniciosus* as a possible vector of sand-fly fever, but a survey of Maltese sand flies in 1932 indicated that they were not as closely associated with the disease as *P. papatasi* [Adler & Theodor (18)]. Sand-fly fever has been diagnosed clinically in China [Bolt (23)], where *P. papatasi* does not occur and where *P. chinensis* and *P. mongolensis* are the main sand flies which feed on man. Up to the present, however, *P. papatasi* is the only sandfly which has been investigated and proved to transmit the disease.

REACTION TO PHLEBOTOMUS BITES (HARARA)

In areas where sand flies are common newcomers from northern countries often develop a skin condition resembling urticaria, called harara in Israel.

Dostrowsky (34), on clinical grounds, assumed that this condition was attributable to sand-fly bites because it was restricted to the parts of the body usually exposed to sand-fly bites and because of its seasonal distribution.

Theodor (93) studied the problem experimentally in England on people not previously exposed to sand-fly bites. There is little or no immediate reaction to first bites except the needle-like pain. Several days (8 to 14 as a rule) after the bite a papule or a small blister appears which may persist for several weeks. In later biting experiments on the same person the papules appear sooner, are larger, more inflamed, and irritating. After three to five

biting experiments the whole area round the bites may become inflamed and oedematous together with a rise in temperature. At this stage old bites which had disappeared were reactivated, and the condition resembled an urticaria. In still later experiments the reactions became weaker and resembled those of the earlier experiments, except that the papules appeared earlier. In still later experiments the character of the reaction changed and wheals appeared immediately after the bite. This type of reaction persists for a long time, after which desensitization follows. One person who had been bitten by sand flies 20 years previously in Mesopotamia gave an immediate wheal reaction.

During the stage of maximum irritation and of reactivation of old bites secondary infection by scratching obscures the original picture, the condition locally referred to as harara is produced, and medical aid is generally sought. This condition has also been recorded from Hungary [Szentkiralyi & Loerincz (91)].

The above description clearly indicates that an allergic process (sensitization) is taking place which is followed by a slow process of desensitization. Harara can thus be defined as the reaction to sand-fly bites at the height of sensitization.

Cases of harara, like cases of sand-fly fever, have not been diagnosed lately in areas in which modern insecticides have brought about the nearly complete disappearance of *P. papatasi* from human habitations.

Since the above paper went to press, Pringle [*Bull. Endemic Diseases, Baghdad*, **1**, 275 (1956)] recorded 24 cases of kala-azar from an unusual type of focus in Iraq. The cases were mostly in children. They were scattered among isolated rural communities in the more arid tracts of the plains around Baghdad. No dogs were found infected, and Pringle suggests an animal reservoir other than the dog. No sand flies of the major group were found in spite of intensive collecting in the infected localities. Pringle thinks *P. papatasi* to be the most likely vector, but this is based only on the epidemiological evidence available and further investigations are needed.

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GENETICS OF INSECT RESISTANCE
TO CHEMICALS[1,2]

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The wide occurrence in nature of resistant insects after several generations of insecticide application is at once an example of rapid evolution and an economic problem. Heritable resistance has been recognized since 1914 when Melander first asked "Can insects become resistant to sprays?" (82). In the first edition (1937) of his influential book, *Genetics and the Origin of Species*, Dobzhansky (36) conceded that evolution is ordinarily so slow that changes within a human lifetime cannot be seen in wild species. But he noted as a conspicuous and important exception to this the development of resistance to cyanide sprays by the California red scale, saying "The spread of the resistant strains constitutes probably the best proof of the effectiveness of natural selection yet obtained." Since that time DDT resistance has appeared and has been extensively studied, but all the evidence still supports the original view: Insecticide resistance is an example of evolutionary change, the insecticide acting as a powerful selective sieve for concentrating resistant mutants that were present in low frequencies in the original population.

This review deals only with genetic aspects of resistance. Comprehensive reviews of insecticide resistance in general up to 1951 have been given by Babers & Pratt (2, 3) and Brown (10). More recent reviews are also available (23, 40, 47, 48, 51, 56, 84, 85, 119).

Is Resistance Pre- or Postadaptive?

High levels of resistance ordinarily occur only where there is a history of exposure to the poison. This immediately raises the question of the role of the poison in the development of resistance. There are two essentially different (though not mutually exclusive) explanations: (*a*) Postadaptation: The change to resistance is physiological and does not depend on the genetic constitution, or if the change is genetic, it is induced directly by the poison. (*b*) Preadaptation: Genetic differences in resistance already are present in the population, and the poison acts as a selective agent favoring the resistant genotypes.

Drug resistance in bacteria.—It is interesting that, while entomologists have almost universally accepted the preadaptive explanation, bacteriologists have frequently assumed postadaptive mechanisms. The problem is a

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[1] The survey of literature pertaining to this review was completed in June, 1956.

[2] Throughout this chapter the symbols R for resistant and S for susceptible are used to designate both strains and genetic factors, in the latter case italicized.

[3] Paper number 635.

subtle one. Because resistance occurs as a consequence of exposure to the drug, it is difficult to show that the drug did not specifically induce it.

The first clear evidence of the preadaptive nature of virus resistance in bacteria was from Luria & Delbrück (77), who showed that the variability in number of resistant cells in replicate clones was too great for consistency with a simple postadaptive hypothesis. The method was immediately applied to drug resistance with similar results. This test is not completely convincing for there is the (perhaps remote) possibility of uncontrolled environmental differences between cultures. More convincing indirect selection experiments have since been done by the Lederbergs and Cavalli-Sforza (22, 66). These embody the same principle as sib or progeny selection in animal and plant breeding (67, 78). Cells were selected on the basis of the resistance of closely related cells, and as a result a resistant culture was obtained where none of the direct ancestors had ever been exposed to the streptomycin. This demonstrated that exposure to the drug is not a necessary condition for the development of resistance, provided there is some method of discovering and concentrating the resistant cells which exist in low frequencies in the original population.

Although this type of analysis has been applied to only a few instances of drug resistance in bacteria, the absence of evidence to the contrary argues strongly for a genetic (preadaptive) rather than a physiological mechanism as the usual cause of drug resistance (for reviews see 13, 21, 34).

Evidence for the preadaptive nature of insecticide resistance.—Because the preadaptive explanation is so widely accepted by entomologists very little attention has been given to testing it specifically. Despite this there is considerable incidental evidence for its validity.

One kind of evidence comes from the repeated failure to demonstrate directly acquired resistance to insecticides. Beard (8) treated milkweed bugs and wax-moth larvae with sublethal doses of nicotine, arsenic, DDT, and pyrethrum. In no case did the treated individuals become more resistant, though in some cases they became more susceptible, possibly as a result of carry-over of the compound or lack of complete recovery. Similar results were reported for DDT in houseflies by Hoffman *et al.* (50) and in the cockroach and milkweed bug by Chang & Crowell (24). Burns (31) grew drosophila[4] larvae in a medium with a sublethal level of DDT or BHC and tested the adults for resistance. Comparisons were made with both DDT-resistant and susceptible strains, but in neither case was there any significant difference between those raised in treated medium and the controls. These studies all agree in showing no effect of pretreatment in conferring an immunity on the insect receiving the treatment (but cf. 39). I am not considering increased tolerance after immediate pretreatment with some fumigants, "protective stupifaction," which is not the result of acquired immunity.

[4] In this article "drosophila" will be used as a common name for *Drosophila melanogaster* Meigen.

There still is a possibility that continued sublethal treatments over many generations might have an effect. But Luers (76) grew drosophila for more than 50 generations in a medium containing a sublethal concentration of DDT and obtained no detectable enhancement of resistance. Thus nonselective doses do not appear to increase resistance.

Luers (76) also tested DDT for mutagenicity, using the Muller-5 method of detecting X-chromosomal lethal mutations. The treated group consisted of flies treated as adults, flies treated throughout larval development, and flies whose ancestors for 50 generations had been exposed to DDT in the culture medium. None of these differed from the controls. It might be argued that this does not show nonmutagenicity, for the DDT may not have reached the appropriate site. However, there is evidence that injected DDT does reach the gonads, and in any event this study shows that DDT as ordinarily used does not have a significant effect on the mutation rate, which is the point at issue. Similar results with DDT and BHC are reported by Wilkes, Pielou & Glasser (120).

Further evidence comes from the failure to attain any appreciable change in resistance from selecting within inbred lines. Such experiments have been done in drosophila by Merrell (83) and Schwartz (31). These experiments are strong support for preadaptation, since there is no reason to think that direct effects of the insecticide would be any less effective in isogenic than in heterogeneous strains. Further, this shows that selection must act mainly on the supply of genetic variants already in the population at the time the selection program begins, not on mutations that occur during the process of selection. There is considerable natural variation in resistance in different drosophila cultures not previously exposed to insecticides (7, 31, 110).

For all these reasons the preadaptation hypothesis seems well established, and we may assume that the sole effect of the insecticide is as a selective agent. It may seem to be beating a dead horse to have emphasized this point, but it is my belief that even the most widely held and reasonable ideas must be continuously held up for questioning (for a postadaptationist view, see 39).

EXTRA-CHROMOSOMAL FACTORS

Hereditary resistance having been established, there arises the question of whether the resistance is attributable to chromosomal or extra-chromosomal factors. Although the bulk of known genetic character are chromosomally determined, there is still the possibility that methological difficulties of analysis of non-Mendelian inheritance have exaggerated the role of the chromosomes (109, 121).

Insects, with the large variety of intracellular symbiotes found in various species, might be especially favorable material in which to find evidences of extra-chromosomal inheritance. In some cases such entities are detectable by effects on the phenotype, the best studied example being the CO_2-sensitive character in drosophila caused by a hereditarily transmitted or infective agent, σ (69, 70). Rasmuson (102) has reported a cytoplasmically

influenced ether sensitivity, and there are well analyzed cases of extra chromosomal influences on sterility in mosquito strain hybrids [Laven & Kitzmiller (61, 63, 64)].

The first suggestion of extra-chromosomal inheritance is a difference in reciprocal crosses, but this may be a result of sex linkage or maternal (and conceivably, paternal) influences. Sex-linked factors result in identical progeny in the homogametic sex but differences in the heterogametic sex in reciprocal crosses and thus are easily recognizable. But distinguishing between a maternal influence, attributable perhaps to delayed phenotypic expression, and extra-chromosomal factors, such as a self-perpetuating cytoplasmic particle, may be difficult, especially if the particle is dependent on a certain chromosome constitution for maintenance (109). For general reviews of methodological and interpretive problems see reviews by Caspari (19) and Lederberg (65).

Genetic analyses of drosophila and house flies, the species for which most information exists, offer no evidence for extra-chromosomal inheritance of resistance. The general rule is that reciprocal crosses give identical results. An exception is reported for DDT resistance in house flies by Pimentel *et al.* (98). The differences between reciprocal crosses were small compared with the differences between the parent strains but were consistent in the two sexes. Since no genetic analysis was made, no conclusion can be drawn about whether the effect is maternal or extra-chromosomal. Johnston, Bogart & Lindquist (55) invoked a cytoplasmic factor for DDT resistance in house flies, but there is no support for this conclusion in their data.

Cochran, Grayson & Levitan (25) report that in the German cockroach the F_1 progeny of R females are slightly more resistant than the reciprocals. The authors suggest that both chromosomal and cytoplasmic R factors are involved, the cytoplasmic factor depending on the chromosomes for maintenance as in *Paramecium* (109). It is necessary to assume that the extra-chromosomal factor is not maintained in the F_1, since the reciprocal difference does not persist to the F_2, and the authors suggest that the maintaining factor may be a single recessive gene in the R strain.

However, the results may equally well be explained by maternal influences, so this cannot be regarded as evidence for extra-chromosomal factors. The material is not very promising for further study, since the difference is small. No reciprocal cross differences in chlordane resistance were found in the same species (41).

Extra-chromosomal inheritance of insecticide resistance would be of great interest and importance for both genetic and physiological analysis. For example, a cytoplasmic particle might be subject to "cure" by chemotherapy, or to transmission by infection or inoculation, or to culture *in vitro*. However, no promising leads have been found in insecticide resistance and, as the next sections will show, those cases where an adequate genetic analysis has been possible have shown the resistance to be entirely attributable to chromosomal genes.

Manner of Inheritance of Resistance

Patterns of resistance in bacteria.—The extensive analyses of drug resistance in bacteria have shown the resistance pattern to be conveniently divided into two classes named after the antibiotics first showing them (13, 21, 34). Resistance of the penicillin type is acquired gradually by the organism, and genetic analysis shows that the resistance is polygenic. The most extensive analysis of this type of resistance was by Cavalli & Maccacaro (20) for resistance to chloramphenicol. In crosses between R and S strains they were able to show that several *R* factors were involved, some being individually identifiable by linkage to chromosome markers.

On the other hand, resistance to very high concentrations of streptomycin frequently comes in one step, as a result of a single mutation. There is almost always polygenic variability as well, but the distinction is that in the streptomycin type high resistance can sometimes be attributable to a single mutant. The most important generalization, from the standpoint of possibly useful analogies for insecticide resistance, is that the pattern of resistance seems to be more a property of the drug than of the bacterial species (13, 21, 34).

Drosophila.—Despite the uncertainties of comparing levels of resistance in different species measured by different methods (15, 29, 46) we can be quite sure that drosophila has not yet developed the extremely high level of resistance that characterizes some strains of house flies. Also, there is no evidence for specific detoxifying mechanisms. One preliminary test (14) failed to show the presence of any dehydrochlorinating enzyme found in many resistant house flies. So the drosophila results are less interesting from some standpoints than house-fly data, but of course a much more precise genetic analysis is possible.

Tsukamoto & Ogaki (91, 114, 115) have studied larval resistance to DDT and other insecticides. They found sizable differences between various laboratory and wild strains, two wild strains (from Hikone and Fukuoka) being especially resistant. Their procedure was to grow mixed cultures in a medium with an appropriate concentration of DDT and count the proportions of different types emerging as adults, the different genetic types being identified with mutant markers. Detailed analysis, using multiply marked strains for crossover detection showed that a single dominant *R* factor, located at about 66 on the second chromosome, was responsible for all the detectable resistance.[5] Tests with BHC showed the same region to be effective, and the authors concluded that DDT and BHC resistance are attributable to the same factor.

In addition to their DDT and BHC resistance, the Hikone strain larvae

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[5] With the limited procedures available in this study it is clearly impossible to distinguish between a single gene (whatever the term means now with increasing knowledge of chromosome fine structure) and a closely linked cluster. The same limitation holds for other cases of "monogenic" inheritance discussed in this chapter.

are resistant to parathion, malathion, and nicotine sulfate. Most of the nicotine resistance is attributable to a region near the centromere of the third chromosome, though other parts of this and the second chromosome seem to have a minor influence. Therefore this resistance is not a result of the same factor as the BHC and DDT resistance (113).

Adult resistance to DDT in drosophila is polygenic if my strain is typical (30). This R strain was developed by growing a large, mixed laboratory population in a cage whose inside surfaces were painted with irregularly increasing amounts of DDT, the idea being to simulate nature as much as possible (29). The adults were tested by exposure for 18 or 24 hr. to DDT residue on filter paper. The crosses utilized S strains with genetically marked chromosomes. All the backcrosses were made so that all heterozygous flies were males, and therefore crossing over between R and S chromosomes cannot complicate the results (105).

The results are illustrated in Figure 1. From inspection it can be seen that the proportion of survivors increases with the number of chromosomes from the resistant strain. Statistical analysis (38) reveals that each of the major R chromosomes makes a significant contribution to resistance.
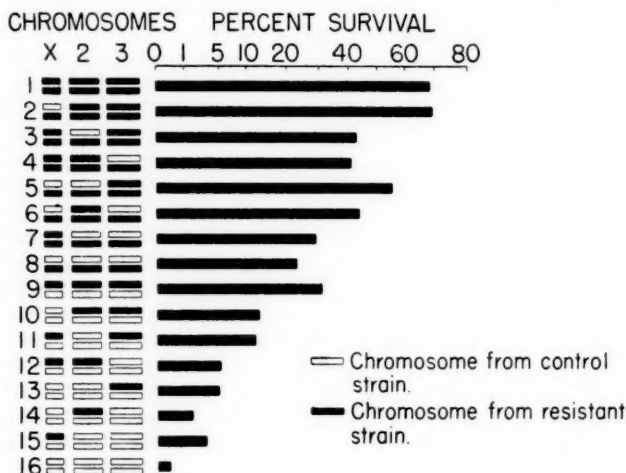
FIG. 1. Polygenic inheritance of DDT resistance in drosophila.

The analysis has been carried a bit further. Tests using crossover markers have shown that most of the dominant resistance in the second chromosome is concentrated in two chromosome regions close to the centromere on either side of it. The precision of the analysis is not sufficient to say whether there is a single locus in each region or several. Likewise it is uncertain how much

resistance is contributed by the rest of the chromosome, but it appears slight compared with the two major regions. Analysis of the dominant factors on the third chromosome led to less conclusive results. All that can be said with assurance is that the results are not consistent with a single chromosome region contributing the major share of the resistance.

Thus we conclude that each of the chromosomes contributes to the resistance, and that it is likely that there are several genes on each chromosome; that is, resistance in this strain is polygenic.

Similar results were obtained by Oshima (93) who by methods similar in principle to those just described, showed that adult resistance in the Hikone strain depends on factors on both major autosomes, though the second seems more important. The studies of King (58, 59, 60), Bochnig (9), and Nachtsheim & Luers (89) are consistent with polygenic inheritance, but no chromosome assays have been published so the interpretation is uncertain.

The genetics of BHC resistance in adult drosophila has been studied by Dresden & Oppenoorth (37, 92) who selected resistant strains by exposing adults to filter paper with BHC deposits. As with DDT, the inheritance appears to be polygenic since no single chromosome could be shown to be responsible for the major share of the resistance.

Oshima & Hiroyoshi (95) have also analyzed a DDT resistant strain of *Drosophila virilis* Sturtevant. Their analysis was for dominant factors only, but they found that all the detectable effect was in two of the chromosomes. Since only two of the five chromosomes appear to be important, this suggests that a rather small number of factors is involved, possibly as few as two.

Cyanide resistance in the California red scale.—This classic case was first discussed by Quayle (99, 100, 101), and the detailed genetic analysis is by Dickson (35). The males die rather soon after mating so the tests were made on females. Over 100,000 insects were tested under carefully controlled conditions, and the data are in excellent agreement with a sex-linked *R* factor. The percentage of survivors for two doses, the second being twice the first, are given in Table I. The only other genetically analyzed case of cyanide re-

TABLE I

PERCENTAGE OF SURVIVORS FROM CYANIDE FUMIGATION IN PROGENY OF
RESISTANT AND SUSCEPTIBLE CALIFORNIA RED SCALE

Parents	Dose A	Dose B
R	45.4	22.4
S	4.1	0.8
R♀×S♂	31.5	13.1
S♀×R♂	33.4	12.4
(R♀×S♂)♀×(R♀×S♂)♂	38.2	17.8
(S♀×R♂)♀×(R♀×S♂)♂	33.9	18.4
(R♀×S♂)♀×(S♀×R♂)♂	17.2	7.0
(S♀×R♂)♀×(S♀×R♂)♂	17.9	7.5

sistance is in drosophila. Harrison (43) was able to reduce the mortality from 90 per cent to 20 to 25 per cent after 46 generations of selection. All or nearly all the resistance is a result of the second chromosome, possibly a single locus.

Difficulties in genetic analysis of resistance. There are several complicating factors that make it very difficult to distinguish monogenic from polygenic factors except in drosophila. It is customary in quantitative characters to assume that any trait that does not lead to Mendelian segregations is polygenic. I suppose that polygenic means any number of factors larger than can be individually identified, which may mean only a few though the number is usually assumed, often gratuitously, to be large enough to permit statistical manipulations based on a normal distribution (68, 78).

Wright (122, 123) has shown by a careful analysis that the amount of white spotting in guinea pigs which superficially looks to be polygenic is in fact largely determined by a single factor. He has also shown how a continuously variable polygenic character with a threshold can simulate monofactorial ratios.

If a single factor with highly variable expression can appear to be polygenic in a character where measurements can be made, it could be even more easily misinterpreted in a trait like resistance where the individual response is all or none. Analysis of such characters is especially difficult (104).

The best method of isolating a major factor, unless linkage tests are feasible as in drosophila or *Escherichia coli* (Migula), is by repeated backcrossing with selection (122, p. 28). If, for example, resistance can be maintained with mild selection over several generations of backcrossing to an S strain, there is probably a major gene. As far as I know this simple procedure has hardly been used at all in resistance work, an exception being an experiment by Busvine (16) who separated DDT from BHC resistance this way. Contrariwise, perhaps the best evidence for polygenic inheritance is the repeated failure to isolate a major gene this way.

Resistance in house flies.—From the studies on DDT and BHC resistance in adult drosophila and HCN resistance in the red scale and in drosophila there is a suggestion of a penicillin and streptomycin pattern as in the bacteria, with resistance to the chlorinated insecticides being polygenic and to cyanide being monogenic. There appeared to be some support for this in the earlier published reports on house fly which were suggestive of polygenic inheritance (11, 28, 62). However, this is quickly dispelled by an examination of newer house-fly data, which in some cases shows quite convincingly that a high level of resistance can be due to a single factor, though there is probably always a background of polygenic variability leading to minor differences in resistance.

Most of the instances where polygenic inheritance of resistance in house flies has been reported (11, 32, 62, 90, 98, 103) are based largely on the gradual acquisition of resistance and the absence of clear-cut monofactorial ratios. As mentioned before, with errors of measurement, environmental variability, and an all-or-none measurement, a gene with a major effect may

very well not be disclosed by the kinds of techniques used. Clear evidence of monofactorial resistance to knockdown in an Italian strain was reported by Harrison (44) who obtained excellent agreement with the expectations based on a single recessive factor for resistance. However, in this case resistance to paralysis is not the same as resistance to the killing effect of the poison (15, 16), and the latter did not lead to simple Mendelian ratios (45, 46). However, Milani (86) found that knockdown resistant strains were also resistant to killing.

Clear evidence for a major dominant gene for high resistance to knockdown (in this case related to resistance to kill) is given by Maelzer & Kirk (81). Their R stock, derived from the Illinois line, consisted mostly of heterozygous individuals, leading the authors to suggest that the homozygotes for the R factor are somehow at a competitive disadvantage. There are some homozygotes in the resistant population, however, for a few flies gave 100 per cent R progeny when crossed with S strains. Lichtwardt, by inbreeding flies descended from the same Illinois strain, was able to fix the R factor and show that the major share of the resistance is attributable to this single factor (72, 73). She further showed that a number of wild resistant flies collected in Illinois carry a dominant R factor and that it very probably is allelic to the R factor in her inbred line (74).

Somewhat different results are reported by Keiding & Barbesgaard (6, 57) from their studies on Danish flies. These strains were from resistant natural populations and had in addition a few generations of laboratory selection. Analaysis of DDT resistance suggests strongly that there is a major recessive factor, though there is considerable background variability. Studies on BHC and chlordane resistance were inconclusive. In neither case was there evidence of a major factor. Busvine & Khan (18) studied F_1 and F_2 hybrids between R and S strains for BHC. There was no evidence for a major factor, but no strong test was made.

From all these studies we conclude that DDT resistance in house flies is probably not the same in various strains, a result not at all surprising in terms of the various possible ways in which resistance can occur. The pattern seems to be somewhat like streptomycin resistance in bacteria; occasionally there is a single mutant that confers a high level of resistance, but it is likely that there is a great deal of polygenic variability in resistance as well, and the accumulation of several such factors in one strain may result in a high level of resistance. However, the last possibility has not been conclusively demonstrated, while high monofactorial resistance has been.

Other genetic studies.—Taylor & Smith (112) have studied crosses between R and S strains for malathion in spider mites [*Tetranychus cinnabarinus* (Boisduval), *T. telarius* (Linnaeus)]. In these species fertilized eggs develop into females and unfertilized eggs into haploid males. Their results are consistent with the interpretation that resistance is a result of a single dominant factor. The F_1 males show strictly matroclinous inheritance of resistance, as they should in a haploid arrhenotokous parthenogenetic species. The back-

crosses show the 1:1 ratio of R to S types expected on a monofactorial hypothesis.

Hough (52, 53, 54) has studied arsenic resistance in the codling moth. In crosses between R and S strains, the F_1 are intermediate as are the F_2. The backcrosses also fall about halfway between the F_1 and the corresponding parental type.

In mosquitos there are numerous studies on resistant strains, but no detailed genetic analysis (for review see 61).

GENETICS OF NATURAL RESISTANCE

There are several instances of insect species which are naturally resistant to various compounds, but there has been very little genetic analysis. One striking species difference in the *D. virilis* group has often been noted by geneticists. *D. virilis* is much more resistant to ether than *Drosophila americana* Spencer. Stern *et al.* (110), by crossing *americana* females with *virilis* males and repeatedly backcrossing the female hybrids to *virilis* males for about two years, obtained a strain in which the cytoplasm was from *americana* and the chromosomes from *virilis*. Such flies were like *virilis* in response to etherization (as well as to several morphological and behavioral traits) showing that the species difference resided in the chromosomes and not in some self-perpetuating extra-chromosomal factor.

I have done a detailed analysis of ether resistance in the two strains (27). By using strains in which the chromosomes were marked by mutants, it was possible to show that the resistance increased with the number of chromosomes of *virilis* origin. Therefore the character is polygenic. The resistance is not specific for ether, as *virilis* is also more resistant to chloroform, carbon disulfide, and DDT.

THE PROCESS OF SELECTION FOR RESISTANCE

Rate of increase in resistance.—The rate of progress by selection ordinarily depends on the amount of heritable variance in the population and the intensity of selection. The quantitative relation between selective intensity and rate of progress is complex and depends on the number of genes involved, dominance and epistasis, amount of environmental effect, counteracting effects of natural selection, etc., but qualitatively the more intense the selection, the more rapid the progress (28, 42, 68, 78). Very few studies have been made to study in detail the rate of progess at varying selection intensities, but in several instances such results were obtained incidentally. Generally the results are as would be expected; the greater the proportion killed each generation, the more rapid the increase in resistance (43, 110).

An exception to this is found in the careful studies of King (58, 59) on DDT resistance in drosophila, and the exception is especially interesting because it illustrates the other part of the principle. He found more rapid progress with 50 per cent mortality than with 95 per cent or higher. I think there

is a ready explanation for this. In his higher intensity of selection the number of survivors was very small, sometimes as few as two flies, and as a consequence the population lost much of its genetic variability. Also, as mentioned earlier, selection within inbred lines is ineffective. In general rapidity of progress will increase with intensity of selection, but only if the number of survivors is large enough to maintain the genetic variability.

It is frequently found that increase in resistance is slow at first, with more rapid increase later (33, 79). An accelerating rate of increase is to be expected on a number of hypotheses. It is likely that the scale is not uniform at various dosages; that is, an equivalent genetic or physiological change may bring a greater change in LD_{50} at some doses than at others. This is especially true for topically applied DDT in acetone (15) or contact treatment (29, 87). Very flat dosage-response curves probably have this explanation (29, 51, 79, 87). It is quite often assumed that a proper metric is the logarithm of the dose, but hardly ever is this assumption tested.

However, there is also a purely kinetic reason for an accelerating rate. The rate of change in gene frequency is proportional to $q(1-q)$ for a gene with no dominance [$q(1-q)^2$ if the R factor is dominant, or $q^2(1-q)$ if recessive] where q is the frequency of the R gene (42, 71). Most factors for resistance must exist in the original population in very low frequency. Clearly the rate of progress can increase enormously as q changes from a very small value (perhaps .001) to a moderate value (.1 or higher). So an accelerating curve of increasing resistance is clearly to be expected, even with a fairly large number of genes involved.

Likewise, as the R genes become less rare the variance in resistance becomes greater, being proportional to $q(1-q)$ for a gene lacking in dominance, or to $q^2(1-q^2)$ if completely dominant. Thus, in addition to an accelerating rate of increase in resistance there should also on this model be an increased variance in resistance (28). If the resistant factor becomes more common than its allele, the variance and rate of change will begin to decrease, but this is likely to happen only if resistance depends on one, or at most a very few genes. The subject of changes in variance, as reflected by the slope of the dosage mortality curve, is discussed by Hoskins & Gordon (51).

Plateaus.—Sometimes, despite rigorously continuing selection, there is no longer any increase in resistance (33, 75, 80). The simplest explanation is that the R factors are homozygous. This probably does not often happen in nature, but some laboratory selections, especially if accompanied by inbreeding, may approach this (72, 75, 124).

However, a plateau is frequently encountered where genetic heterogeneity is high, a situation much discussed (26) and recently reviewed by Lerner (68). An example is given by Robertson (26) where selection for bristle number reached a plateau in several drosophila stocks. The explanation turned out frequently to be a lethal effect such that the homozygotes for high bristle number factors were largely eliminated by natural selection. Such a

situation would not ordinarily be detected, except in drosophila. In insecticide resistance the most resistant genotypes are frequently less viable, or develop more slowly (96, 118), or are less fertile (6), or the most resistant genotype may be a heterozygote rather than a homozygote.

It is interesting to view the findings of Maelzer & Kirk (81) and Lichtwardt *et al.* (74) in this light. In both cases, one a noninbred laboratory population, the other a natural population, the dominant *R* factor had remained in an unfixed condition. Only by rigorous inbreeding was a homozygous population achieved, not by selection alone (72). This seems to fit the sort of situation discussed by Lerner (68) and Robertson (26). Somehow the homozygote must be less fit, so that in natural competition it is regularly eliminated.

Return to susceptibility with relaxed selection.—Lerner (68) has said,

Attempts to shift populations too rapidly and too far from adapted mean values for specific traits, either by artificial selection or by changes in the breeding system, are counteracted by natural selection which is directed towards the maintenance of a phenotypic balance between fitness-determining characters. This behaviour is a product of the previous evolutionary history of the population.

Since the genes causing insecticide resistance were at low frequency in the population before the insecticide began to be applied, it must ordinarily be true that they are to some extent disadvantageous; otherwise they would have been common. Therefore the selection for resistance should ordinarily involve the replacement of the original genes with *R* factors that, in every respect except insecticide resistance, are deleterious from a survival standpoint. One should then expect that when the culture is grown in the absence of the insecticide it will return to susceptibility. This return may, of course, be very slow if the factors are only mildly disadvantageous.

Regression to susceptibility when selection for resistance is relaxed is often observed (6, 96, 97, 98, 118) though sometimes not (29, 73, 75, 94). One would expect that when selection for resistance is accompanied by a great deal of natural selection for general fitness the only kind of resistance factors that would become frequent in the population would be those that cause very little reduction in fitness. Strains developed in this way would return to susceptibility very slowly when removed from the insecticide. On the other hand intense selection for resistance under circumstances where there is very little natural competition (e.g., selection in uncrowded laboratory cultures) some of the flies may be highly resistant, but pretty poor specimens for natural survival otherwise. These would revert to susceptibility much more rapidly. It is hard to judge from the published literature whether this rule is being followed, but there are some examples in agreement (98).

Drosophila populations do not appear to have the extremely high resistance that some house-fly strains do, and the resistant may be largely what Hoskins & Gordon (51) call vigor tolerance. In my strains (29) there was no perceptible decrease in resistance after three years of no selection. These flies

had been selected for DDT resistance under circumstances of severe natural selection in population cages, so one would expect that only R factors of near normal viability would be selected.

There are three possibilities. One is that the resistance factors were homozygous, and hence there was no reversion (75). This can be quickly tested, and the experiments were done by Burns (31). Inbred lines were made from the resistant strains and then crossed. The various F_1 hybrids showed considerable variability in resistant between crosses, in fact about as much variance as between inbred lines derived from nonresistant strains. So the population is clearly not homozygous.

A second possibility is that the resistance factors enter into new kinds of balanced combinations with other factors acting as viability modifiers, so that the genotypic structure of the population is so altered that the formerly deleterious factors, now incorporated into the population, have been made favorable. No doubt such processes occur in the long run of evolution, though it may be doubted whether that would happen within the short course of a laboratory selection experiment. A ready test of this hypothesis exists, however. Morton (31) tested the reversion rates of hybrids between two different resistant populations, and hybrids between resistant and susceptible populations. The rationale for this test is that the various integrative combinations that might have been built up would surely not be identical in the two populations and would be broken up by recombination in the hybrids. However the hybrids showed no measurable regression either. A very similar experiment, and with very similar results, was reported by Oshima (94).

We are left then with the simplest explanation, that the R factors in this experiment are very nearly neutral. It will be of interest to follow the cultures over a much longer time and see if there is an eventual return to susceptibility.

Since selection of resistance in nature must always be accompanied by intense natural selection for general viability and fertility it is unlikely that resistance factors will be selected if they are highly detrimental otherwise. Hence reversion to susceptibility should be slow, and one cannot hold out much hope that a short period of absence of the insecticide is likely to lead to a susceptible population. Furthermore, if the population does return to susceptibility it may require a long time for the genes to be carried below a certain frequency, just as it often takes a long time to accomplish the early part of the increase in resistance. This is because selection in either direction is slow when the gene is rare. Therefore a susceptible population that has once been resistant is likely to increase rapidly in resistance when the insecticide is again applied. This has frequently occurred in laboratories where a population that had lost some resistance was quickly built up to its former level by a few generations of selection.

Effective population number and the selection process. I have mentioned earlier that the effective population number has an influence on the effectiveness of selection; if the number is small enough to reduce the amount of

genetic variability, selection is less effective. There is another possible influence of the effective population number, that of influencing the amount of gene interaction in polygenic systems.

One of the striking findings in the analysis of polygenic resistance in bacteria has been the interdependence of the R genes. Cavalli (20) showed that in crosses between R and S strains the recombinant types were often highly susceptible but hardly ever approached the R parent in resistance, a finding readily interpretable as attributable to complementary interaction of the R genes. On the contrary, my studies of drosophila resistance referred to earlier showed an almost complete absence of interaction, some 95 per cent of the variance in the proportion surviving (with a variance stabilizing arcsine transformation) being attributable to additive effects of chromosomes (30). Additional evidence for absence of any significant amount of complementary interaction of R factors is shown by the fact that the F_2 between different R strains does not fall significantly below the average of the F_1 and mid-parent.

The difference has been explained as follows (30). In the clonal bacterial population R mutants occur during the course of the selection and are immediately selected into the population. After one mutant has been incorporated, the next may well be one that increases resistance only in the presence of the first, perhaps a modifier, and so on with subsequent mutants. The immediate incorporation of each new mutant into the population thus favors the building up of interdependent factors. The insect population is quite different. There is relatively slow change in gene frequencies, all or most of the R factors were already in the population before selection started, and the genes are reassorted every generation by sexual reproduction. Under this system the kind of R gene most likely to succeed in being selected is one that produces some resistance in all the variety of genotypes present in the population, i.e., a gene with additive action. Genes with complex interactions, though many may be present in the population, are not selected efficiently and hence remain in low frequency.

King (60, 69) has obtained quite different results in his drosophila experiments. He found that the F_2 of crosses between two different R strains were often quite susceptible and interpreted this as evidence for "integration of the gene pool." The main relevant respect in which King's experiments differed from mine is that he started with a small initial population derived from a few wild flies whereas my original population was large and had been deliberately made heterogeneous. A relatively homogeneous population would be more like a bacterial clone and would be more likely to favor the selection of genes that function in a specific background genotype. Since this background varies from strain to strain the resistance system would tend to be broken up in strain crosses. Thus the general hypothesis (fortunately, easily testable) is that complementary interaction is more likely to develop when the effective population number is small.

CORRELATES OF RESISTANCE

Resistance to other insecticides.—Numerous instances are known of strains resistant to several insecticides, often substances not apparently related (12, 17, 51, 84, 87, 97, 113). In some cases these are what Hoskins & Gordon (51) call vigor tolerance, i.e., dependent on nonspecific factors. In a few instances DDT resistant lines have been unusually sensitive to some other substance, but this is probably a property of the particular strain (1) or of the method of selection (111).

Busvine (16) started with a multiply resistant strain, and by selecting for resistance to one poison while repeatedly backcrossing to an S strain he was able to separate DDT resistance from BHC resistance. Thus different genes are involved, as might have been expected on biochemical grounds. Similarly, Barbesgaard & Keiding (6) reported that a strain segregating for DDT resistance was uniformly resistant to BHC. As mentioned in an earlier section, repeated backcrossing is perhaps the best way of isolating a major factor if one exists, and the fact that Busvine was able to retain resistance with rather mild selection during repeated backcrossing and to effect such a complete separation of types of resistance probably means that very few genes were involved. In Busvine's experiments BHC, chlordane, and dieldrin resistance remained inseparably associated, suggesting the same genetic mechanism for all. This kind of procedure should be very useful for analysis of other multiply resistant strains to see to what extent the resistances are separable.

Correlation of resistance with other characters.—Many instances of morphological, physiological, enzymatic, and behavioral differences between S and R strains probably are of no significance, being simply properties of the particular strains studied and not in general associated with resistance (4, 5). For example, Lichtwardt (72) found a wing anomaly in her inbred R strain of house flies, but in crosses the two characters segregated independently. A detailed morphometric analysis of 16 body measurements showed no relationship of any, or any combination, with resistance [Sokal & Hunter (108)].

On the other hand, some correlations appear to be real genetic associations, probably pleiotropy rather than linkage. Snyder (106) has repeatedly found a wing abnormality in DFP resistant house flies. Hiroyoshi (49) showed that the same region of the chromosome that is responsible for DDT and BHC resistance in the Hikone strain of drosophila results in an excess of iron deposited in the body.

Sokal & Hunter (107) showed that a low level of larval resistance to DDT in drosophila was associated with a behavioral factor, a tendency to pupate at the periphery of the medium. They were able to show that the association was not spurious by doing the reverse experiment; by selecting for peripheral pupation they were able to increase the DDT resistance.

Concluding remarks.—There has not been much use of genetics for studying the physiology and biochemistry of resistance (87). The finding of single

genes that confer a high level of resistance offers a promising possibility for study of specific aspects of resistance. For example, the R gene in the Illinois strain is possibly associated with dehydrochlorination (56, 74). The procedure of backcrossing while selecting for resistance to one compound is a very effective way of isolating a single gene if one exists. This kind of procedure was used very effectively by Busvine (16) to separate DDT from BHC resistance.

It might be useful to select simultaneously for resistance to one substance and susceptibility to another. Selecting for DDT resistance and DANP susceptibility might lead to a strain whose resistance is specifically a result of dehydrochlorinating ability. Another possibility is to select for resistance to simultaneous treatment with an insecticide and its antagonist or analogue. This might greatly raise the enzyme level and facilitate chemical analysis [Moorefield & Kearns (88)]. There has been considerable study of the pharmacology of jointly applied drugs (116, 117), and genetic strains selected for joint resistance might be useful. With as many types of resistance as there are it should be possible to develop a series of strains with specific types of resistance.

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THE MODE OF ACTION OF INSECTICIDES EXCLUSIVE OF ORGANIC PHOSPHORUS COMPOUNDS[1]

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This review deals largely with those papers which have appeared since the subject was reviewed by Kearns (1) in Volume 1 of this series, and is exclusive of the literature pertaining to organic phosphorus compounds. The latter topic is being reviewed in the present volume by Spencer & O'Brien (2) and Bennett (3). In addition, Cutkomp (4) has prepared a review of recent trends in insecticide research which cites 154 references and Winteringham & Barnes (5) have prepared a review of the response of insects and mammals to certain halogenated hydrocarbon insecticides which includes 335 references. And finally, there is the book by Metcalf (6) dealing specifically with the chemistry and mode of action of organic insecticides.

DDT

Metabolism and detoxication.—Chadwick has reviewed recent data pertaining to the biochemical degradation and mode of action of DDT in the insect body (7) and the genetic basis of acquired resistance and various types of physiological mechanisms involved in resistance (8). Kearns (9) has reviewed the significant aspects of enzymatic detoxication of DDT.

Roth *et al.* (10) treated resistant house flies, *Musca domestica* Linnaeus, individually with C^{14}-labeled DDT to determine the amount of DDT absorbed by the flies at different temperatures. The flies absorbed 64 per cent more of the toxicant with a lower mortality during a 24 hr., post-treatment period at 90°F. than when held at 70°F. The excrement of treated flies showed some radioactivity, approximately 15 per cent of the total DDT absorbed was accounted for in the excrement over a seven-day period.

Butts *et al.* (11), using 2-C^{14}-labeled DDT, reported a detoxication product in American cockroaches, *Periplaneta americana* (Linnaeus), injected with the insecticide and speculated that the water-soluble, radioactive product was probably a conjugated compound composed of a derivative of DDT and another fragment, possibly carbohydrate in nature.

Robbins & Dahm (12) studied the absorption and excretion, tissue distribution, and metabolism of p,p'-C^{14}- and 2-C^{14}-ethane-labeled DDT and the tissue excretion and distribution of p,p'-C^{14}-labeled DDE in female, American cockroaches. Topically applied radioactive DDT and DDE were rapidly absorbed and widely distributed internally. As much as 75 per cent of the DDT applied was excreted as metabolites in the feces over a 24-day period. About 80 per cent of the radioactivity in the feces was attributable

[1] The survey of literature pertaining to this review was completed in June, 1956.

to metabolites containing the diphenyl-2-carbon moiety of DDT; less than 10 per cent was a result of DDT, DDE, or DDA. When piperonyl cyclonene was used with DDT, it inhibited absorption of DDT and excretion of metabolites.

Cochran (13) studied the distribution and sites of metabolism of DDT injected into the hemocoele of the American cockroach. The DDT was widely distributed within the insects; a conclusion which was also reported by Robbins & Dahm (12). Metabolism of DDT and DDE apparently takes place in a number of tissues, and in adult cockroaches. DDE was found in the greatest quantities in the fat body, alimentary canal, and remaining tissues. It was concluded that the fat body is important in relation to the differential susceptibility to DDT exhibited by the sexes and developmental stages of this insect.

The absorption, metabolism, and excretion of p,p'-C^{14}-labeled DDT in adult, female Madeira roaches, *Leucophaea maderae* (Fabricius), and fifth instar European corn borer larvae, *Pyrausta nubilalis* (Hübner), were studied by Lindquist & Dahm (14). The Madeira roach absorbed DDT rather slowly and excreted 50 per cent of the total applied radioactivity over a period of 36 days. The presence of DDT, DDE, and three metabolites in the feces was identified by paper chromatography. DDT was the predominant radioactive compound excreted the first 24 hr. after treatment, after which an unidentified metabolite was the major radioactive compound excreted. Several metabolic pathways for DDT in the Madeira roach are proposed. Fifth instar European corn borer larvae possess some tolerance to DDT and convert significant amounts of absorbed DDT to DDE. No evidence of other DDT metabolites was found.

Perry *et al.* (15) studied carefully the degradation products of DDT in seven DDT-resistant strains of house flies, using C^{14}-labeled DDT. The only significant product of DDT metabolism was DDE. Both DDT and DDE were found in the ether-soluble portion of the excreta, the DDE-DDT ratio increased with time. Very small amounts of a radioactive product were found in the water-soluble portion of the excreta. No strain specificity was evident. In flies held 10 days after application of the insecticide, small but consistent losses of DDT were experienced which might be attributed to incomplete recovery of material from excreta.

Resistant and susceptible house flies have been examined by Terriere & Schonbrod (16) for evidence of metabolism of DDT up to 14 days after topical treatment with C^{14}-tertiary carbon-labeled DDT. Flies from the susceptible strain excreted up to 88 per cent of the applied DDT in the form of a water-soluble conjugate. The DDT-resistant flies showed a similar detoxication and excretion pattern. The metabolic conjugate was hydrolyzable with acid to produce a compound weakly acidic in nature.

Perry & Sacktor (17) examined ten strains of susceptible and DDT-resistant house flies for their ability to convert DDT to DDE, to absorb DDT, and for their cytochrome oxidase activity. The susceptible strains metabo-

lized little DDT whereas the resistant strains converted DDT to DDE, the amount varying with each strain. Some strains also differed in the rate of absorption of topically applied DDT. There was no direct relationship between cytochrome oxidase activity and degradation of DDT in the various strains. This study emphasizes again that DDT resistance cannot be characterized by a single common factor; apparently, each strain possesses a combination of attributes for resistance which may be different from that found in other strains.

Effects on enzymes.—The effect of different concentrations of DDT on the activity of cytochrome oxidase in homogenates of the yellow mealworm, *Tenebrio molitor* Linnaeus, and isolated leg muscles of *P. americana*, using both spectrophotometric and manometric procedures, was studied by Ludwig *et al.* (18). Inhibition of cytochrome oxidase appeared in all experiments with 10^{-3} M DDT, the amount varying with the time of incubation at room temperature. These experiments add further evidence to the theory that DDT may poison the insect through its effects on cytochrome oxidase.

The effects of certain insecticides on cholinesterase obtained from homogenized brain tissue of adult American cockroaches were studied by Hartley & Brown (19). A total of 32 insecticidal compounds, including chlorinated hydrocarbons, dinitro and organic phosphorus compounds, botanically derived insecticides and miscellaneous organic compounds, were tested by two manometric methods. None of the 13 chlorinated hydrocarbon insecticides tested had a significant anti-enzyme effect. As would be expected, several of the organic phosphorus compounds and a carbamate exhibited anticholinesterase effects. Nicotine also was a partial inhibitor of this enzyme.

Frontali (20) was able to detect no inhibitory action on cholinesterase when DDT was added *in vitro* to head homogenates of susceptible and resistant house flies. Anderson & March (21) have concluded that inhibition of insect carbonic anhydrase cannot be an important factor in the mode of action of DDT or other organic insecticides.

Structural relationships and synergism.—An interesting structural and insecticidal relationship between rotenone, methoxychlor, and DDT has been presented by Hummer & Kenaga (22). Riemschneider (23) examined the stereochemistry and contact toxicity of DDT analogues using Stuart models and concluded that a parallelism exists between the degree of free rotation and the efficacy of contact action.

Bellemare & Belcourt (24) have injected the cyanide analogue of DDT, 2,2-bis(*p*-cyanophenyl)-1,1,1-trichloroethane, into the American cockroach. In low concentrations this compound stimulates the motor fibers in a reflex manner by producing, at the ganglionic level, waves of impulses from the afferent nervous fibers; in stronger doses, it may act directly upon the motor elements without having recourse to the reflex arc. Since these results are identical to those which others have obtained for DDT, one can conclude that DDT and its cyanide analogue have a similar mode of action.

Tahori (25) tested 29 compounds structurally related to DDT as DDT synergists against resistant strains of the oriental house fly, *Musca domestica vicina* Macquart; bis-(*p*-chlorophenyl)-trifluoromethyl carbinol was the most active synergist. Synergistic activity was reduced by replacement of the chlorine atoms by other halogens, alkyl, nitro, and alkoxy radicals, by reduction of the carbinol to the corresponding ethane, by its esterification, by a prolongation of the aliphatic chain, or by elimination of one *p*-chlorophenyl group.

Physiological and morphological considerations.—DDT has been used by Hodgson & Smyth (26) to study the localization of certain sense organs in the flesh fly, *Sarcophaga bullata* Parker. Weiant (27) demonstrated that sensory nerves of DDT-resistant house flies are less sensitive to the direct action of DDT than are similar nerves of susceptible flies. The DDT-resistant flies are also able to recover from and adapt to DDT poisoning more readily. Therefore, a basic physiological difference between the sensory nerves of DDT-resistant and susceptible house flies has been postulated.

Lofgren & Cutkomp (28) have concluded that the quantity of lipides present is not a factor which might be responsible for the negative temperature effect of DDT which occurs when male and female American cockroaches are subjected to contrasting post-treatment temperatures. Furthermore, the differences in quantity of lipides between sexes is not sufficient to explain sex differences in response to the action of DDT.

Sokal & Hunter (29) have concluded that DDT-resistance is not correlated with any of the 16 morphological characters, or four ratios computed therefrom, measured on about 1000 flies from five DDT-resistant and four nonresistant strains of house flies.

BHC

Van Asperen (30) has investigated the mechanism of action of benzene hexachloride isomers using *P. americana* and mice. According to the anti-inositol theory suggested by Slade in 1945, gamma BHC and the B-vitamin, meso-inositol, have identical stereochemical structures, and gamma BHC acts as a metabolite antagonist. Although in some cases the toxic effects of gamma BHC are antagonized by meso-inositol, there is no proof that the latter is an essential metabolite in insects and higher mammals. It is doubtful, however, that this theory is of general validity. Similar antagonistic effects between the different stereo-isomers of BHC were shown to occur in cockroaches and mice proving that the stereo configurations of antagonists need not be identical. The high toxicity of the gamma isomer, in comparison with the rather low toxicities of other isomers, points to the necessity for an exact fit of the gamma BHC molecule on a reacting surface in the animal body. The antagonistic effects exerted by the other isomers suggest the possibility that these isomers fit on the same reacting surface without causing effects detrimental to the animal's metabolism.

Van Asperen (31) has also evaluated the joint action of gamma and delta

and gamma and alpha isomers of benzene hexachloride injected into adult *P. americana*. From the mortalities obtained, it would appear that independent action, with a strong positive correlation of the tolerances for the two poisons, can give an appropriate explanation of the results. It is possible, however, that antagonistic effects also have played a part in the joint action of the isomers. A method of separating the isomers of benzene hexachloride by reversed-phase paper chromatography has been reported by Bridges *et al.* (32).

Other Synthetic Organic Insecticides and Acaricides

Chlorinated hydrocarbon insecticides.—Sumerford (33) has reviewed the literature dealing with synergism among halogen-containing insecticides and halogen-containing synergists.

Cochran (34) has shown that adult, female American cockroaches were normally less susceptible than adult males to the effects of injected known amounts of the following chlorinated hydrocarbon insecticides: lindane, chlordane, toxaphene, dieldrin, methoxychlor, and DDT. With the last instar nymphs, the sexual difference in susceptibility apparently does not hold.

Born & Davidson (35) evaluated the toxicity of aldrin, dieldrin, endrin, isodrin, chlordane, and heptachlor, alone and in combination with pyrethrin, to DDT-resistant and susceptible house flies. All of the compounds gave increased mortality when combined with pyrethrins and tested against flies of the DDT-resistant strain. When the susceptible strain was used, the results were additive for all compounds except heptachlor and isodrin. These two compounds gave indications of antagonism in combination with pyrethrins which might be attributable to physiological differences between the two strains of flies since there was no evidence of antagonism using resistant flies. The resistant strain may show a higher degree of susceptibility to some combinations of these toxicants than does the susceptible strain.

A susceptible and a chlordane-resistant strain of German cockroaches, *Blattella germanica* (Linnaeus), were used by Butts & Davidson (36) to determine the relative toxicities of aldrin, chlordane, dieldrin, heptachlor, and lindane by injection into the hemocoele. The resistant strain of roaches showed some degree of resistance to all the insecticides, but the highest degree of resistance was exhibited to heptachlor.

Lin & Richards (37) reported the absence of any qualitative difference in respiratory enzyme composition between three strains of house flies as judged by temperature kinetic determinations. The strains of flies used included a susceptible strain, a strain resistant to DDT (DMC), and a strain resistant to DDT, BHC, lindane, dieldrin, aldrin, and several other insecticides (Multi-X).

Knutson (38) has shown that sublethal exposures of *Drosophila melanogaster* to dieldrin increased the fecundity and life span of the flies. The greater total progeny of the dieldrin-exposed flies resulted apparently be-

cause these flies lived longer than the control flies and therefore had a longer time in which to reproduce.

Hopkins & Hoffman (39) have shown that piperonyl butoxide, the α-propylpiperonyl ester of propionic acid, and the α-allylpiperonyl ester of senecioic acid, are especially effective as synergists with Dilan. These compounds, in combination with Dilan, were effective against DDT-resistant house flies and stable flies, *Stomoxys calcitrans* (Linnaeus).

Carbamic acid derivatives.—Kolbezen *et al.* (40) have investigated the hypothesis that a number of derivatives of carbamic acids, containing the quaternary nitrogen structure and possessing anti-cholinesterase properties, are not active as contact insecticides because of their inability to penetrate insect cuticle and fatty nerve sheath. Some 49 lipoid-soluble carbamates were synthesized and evaluated as contact insecticides and as *in vitro* inhibitors of house fly brain cholinesterase. The N-methylcarbamates of *m-tert*-butylphenol, thymol, and carvacrol were highly toxic to house flies and greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouché). The more toxic compounds were also tested on the spirea aphid, *Aphis spiraecola* Patch, and the citrus red mite, *Metatetranychus citri* (McGregor). Cholinergic activity and toxicity were inversely dependent on the rates of hydrolysis of the carbamates, and *in vitro* cholinesterase inhibition was related to contact toxicity. The second-order hydrolysis constants for 15 of the carbamates were determined and a relationship was shown between stability to hydrolysis and cholinesterase inhibition.

Assuming that enzymatic reactions involving the hydrolysis and esterification of substrates appear to fall in the general class of acid-base catalyzed reactions, the inhibition of cholinesterase hydrolysis of acetylcholine by substituted phenyl carbamates (I) appears to be one of competition and should satisfy the following criteria: (*a*) the carbamate must possess the structural requirements to "fit well" on the enzyme, (*b*) the carbamate must be reasonably stable to hydrolysis at the site, and (*c*) the carbamate must remain firmly attached to the enzyme by a combination of its conformal fit and other intermolecular forces involved. Structural changes involving R and the substituent X on the reactivity of the carbamate linkage have shown that the order of effectiveness for cholinesterase inhibition is $R = CH_3 > CH_3CH_2 \gg C_6H_5CH_2 > C_6H_5$. Variations in the benzene ring substituent X, R constant, show that the order of increasing inhibitory activity corresponds roughly to the order of increasing electron donating abilities. The enzyme-carbamate complex formation may take place at either the carbonyl or ether oxygen. Inactivation of the carbamate probably takes place by hydrolysis, which is analogous to the acid-base catalyzed mechanism.

Acaricides.—Kenaga & Hummer (41) have evaluated the toxicity of some substituted phenyl benzenesulfonates, and Kenaga (42, 43) has summarized the toxicity of some bis(substituted phenoxy) methanes and some substituted phenyl benzoates to the eggs and adults of the two-spotted spider mite, *Tetranychus telarius* (Linnaeus), and larvae of the Mexican bean beetle,

$$X \underset{}{\bigcirc} - O - \overset{\overset{O}{\|}}{C} - NHR$$
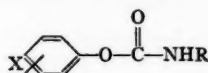
(I) Structure of substituted phenyl carbamates

$$Cl \underset{}{\bigcirc} - \overset{\overset{O}{\|}}{\underset{\overset{\|}{O}}{S}} - O - \underset{}{\bigcirc} Cl$$

(II)

$$Cl \underset{}{\bigcirc} - O - CH_2 - O - \underset{}{\bigcirc} Cl$$

(III)

$$Cl \underset{}{\bigcirc} - \overset{\overset{O}{\|}}{C} - O - \underset{}{\bigcirc} Cl$$

(IV)

$$Cl \underset{}{\bigcirc} - \underset{\overset{|}{O-R}}{CHC} Cl_3$$

(V)
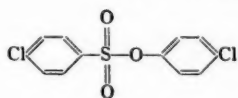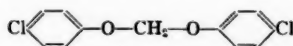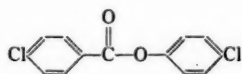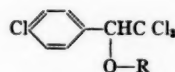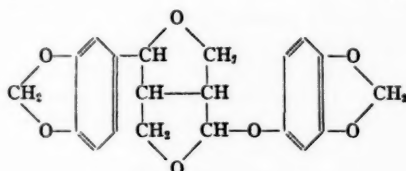
(VI) Sesamolin

Epilachna varivestis Mulsant. Optimum mite ovicidal activity and toxicity to bean beetle larvae occurred with the 4-chlorophenyl ester of 4-chlorobenzenesulfonic acid (II). Bis(4-chlorophenoxy)methane (III) was toxic to all stages of the arthropods used in these tests; ring substitution of 2-allyl,4-methoxy groups resulted in a compound that was toxic to spider mite adults and bean beetle larvae. The ovicidal properties of substituted phenyl benzoates was greatest in the case of the 4-chlorophenyl ester of 4-chlorobenzoic acid (IV).

The acaricidal action of 2,2,2-trichloro-1-(*p*-chlorophenyl)-ethanol suggested further studies on the effect of the hydroxyl group. Fifteen ester and ether derivatives of this compound (V) were synthesized by Kolbezen *et al*. (44) and bioassayed against four species of insects and one mite species. The effects of reducing the polarity of the hydroxyl group upon penetration and transport and the possible hydrolysis of the esters were studied. No marked differences in toxicity were noted in the experiments with the mite and three species of insects, but a specificity of certain compounds was observed for the confused flour beetle.

Barker & Christian (45) have synthesized C^{14}-carbonyl labeled dimethyl phthalate.

INSECTICIDES OF BOTANICAL ORIGIN AND RELATED COMPOUNDS

Pyrethroids and allethrin.—Winteringham *et al.* (46) have studied further the *in vivo* metabolism of C^{14}-labeled pyrethroids and allethrin in adult *Musca domestica* by injecting or applying topically allethrin, the natural mixture of pyrethroids, or the chrysanthemic esters separated chromatographically. Significant and comparable fractions of all the applied pyrethroids were metabolized to relatively noninsecticidal substances within 24 hr. When piperonyl cyclonene was applied simultaneously with the pyrethroid, the metabolism was substantially inhibited, but least effectively in the case of allethrin. This suggested that the synergism involved an interference with the natural detoxication mechanism of the house fly and leaves open the possible development of a more effective allethrin synergist. Absorption of the pyrethroids applied topically was almost complete within 24 hr. and was apparently nonselective from a mixture of esters. The presence of piperonyl cyclonene invariably retarded absorption, presumably by dilution of the pyrethroid on the insect integument.

In pursuance of the mode of action of allethrin, Mitlin & Babers (47) have determined the relative toxicity of topically applied allethrin and pyrethrins to susceptible and resistant strains of female house flies, male German cockroaches, and last-instar nymphs of American cockroaches. Allethrin was as toxic as pyrethrins to both strains of house flies, 0.03 as toxic to the susceptible and resistant German cockroaches, and 0.15 as toxic to last-instar nymphs of the American cockroach.

The excessively wet appearance of house flies dying after treatment with pyrethrum prompted Ingram (48) to study further the water loss from house flies and nymphal American cockroaches treated with pyrethrum. Apparently, neither depletion of energy reserves as a result of hyperactivity, nor loss of water, was the direct cause of death. The water lost through the integument can be explained by the neurotoxic action of pyrethrum on a hypothetical regulatory mechanism involving secretion by the epidermal cells. A similar regulatory mechanism in ticks and spiders has been suggested previously by other workers.

Synergism.—Blackith (49), Turner (50), and Schmidt (51) have reviewed the development and mode of action of synergists for pyrethrins and related synthetic compounds. Matsubara (52) investigated the inhibitory action of piperonyl butoxide on detoxication of pyrethroids by house fly tissue homogenates. The enzymatic detoxication of pyrethroids was inhibited 55 per cent by piperonyl butoxide. Combination of allethrin with piperonyl butoxide resulted in very little enzymatic inhibition suggesting a basic difference in the mechanism of synergism.

Matsubara (53) has concluded that a part of the mechanism of synergistic action in relation to the knockdown effect of pyrethrins on house flies is attributable to inhibition by the synergist of enzymatic detoxication of pyrethrins. The detoxication of pyrethrins by triturated tissue from oriental house flies was totally or largely inhibited by dihydroconiferyl alcohol,

guaiacol, anisol, and phenol. A group of nonsynergistic compounds did not inhibit detoxication.

Nash (54) studied the synergistic effect of piperonyl butoxide and isobutyl undecylenamide (IN-930) on pyrethrins and allethrin. The effects of these synergists were assayed with bed bugs, *Cimex lectularius* Linnaeus, by means of a residual film technique, and with house flies using a measured drop technique. Under these conditions, pyrethrin was 5.5 times as toxic to bed bugs as allethrin, while only twice as toxic to house flies. The two synergists were tested at ratios from 1:1 to 20:1 with the insecticides. Piperonyl butoxide was the more potent synergist, increasing the effect of the pyrethrins five times and allethrin four times to house flies and to a lesser degree in the case of bed bugs. IN-930 did not increase the potency of either insecticide more than twice.

Fales & Bodenstein (55) compared piperonyl butoxide, sulfoxide, *n*-propyl isome, and MGK 264 as synergists for both allethrin and pyrethrins in spray tests against the yellow fever mosquito, *Aedes aegypti* (Linnaeus), and the common malaria mosquito, *Anopheles quadrimaculatus* Say. Allethrin was less effective than pyrethrum against mosquitoes. Against *Aedes*, piperonyl butoxide was the most effective synergist when used with pyrethrins; sulfoxide and *n*-propyl isome also were synergistic in their action, but MGK 264 caused no synergism. With allethrin, the degree of synergism was not as great as with pyrethrins, and MGK 264 again caused no synergism. Against *Anopheles*, the results were similar to those with *Aedes* when the synergists were used with pyrethrins. With allethrin, however, MGK 264 was equal to piperonyl butoxide, and sulfoxide and *n*-propyl isome were slightly more effective. When the synergists were used by themselves, only MGK 264 showed slight toxicity.

Although piperonyl butoxide has been effectively used as a pyrethrum synergist, it has been reported to be only one-hundredth as toxic as pyrethrins when tested against house flies by the turntable method. However, Mitlin & Konecky (56) have discovered that piperonyl butoxide inhibits the development of house flies when it is added to larval medium in concentrations from 0.074 to 0.25 per cent by weight. The length of larval life is directly proportional to the concentration of the chemical, and the percentage of adult emergence is inversely proportional. Death occurs in the third larval instar or early during pupation. Both normal and DDT-resistant strains of flies were affected similarly, but the effect was greater in the DDT-resistant strain. The addition of fly lipase to the larval medium in concentrations up to 0.75 per cent did not overcome the action of piperonyl butoxide, although one possible mode of action of piperonyl butoxide has been ascribed to be that of lipase inhibition.

The importance of the 3,4-methylenedioxyphenyl group for synergism of pyrethrins has been established. The synergism problem has been complicated recently by the introduction of synthetic pyrethrin-like compounds such as allethrin, furethrin, and cyclethrin. The establishment of sesamolin

as one of the most potent natural pyrethrin synergists, and the elucidation of its probable chemical structure (VI), prompted Beroza (57) to investigate compounds containing a 3,4-methylenedioxyphenoxy group in place of one of the 3,4-methylenedioxyphenyl groups of sesamin and related synergists. Of some 66 compounds prepared and tested for synergism with pyrethrins and allethrin against the house fly by the turntable method, the acetals appeared to be the most promising candidates for synergists of commercial value. Although synergism was much greater with the natural pyrethrins than with allethrin, the results were generally parallel.

Blum & Kearns (58) have reported that the toxicity of sabadilla towards the house fly was increased by six pyrethrum synergists; sulfoxide and piperonyl butoxide were the most effective. Blum (59) has utilized the tendency of methylenedioxyphenyl compounds to form colored complexes in the presence of gallic and sulfuric acids to develop a quantitative test for pyrethrum synergists including sesamin, piperonyl cyclonene, piperonyl butoxide, and n-propyl isome.

Schmidt & Dahm (60) prepared C^{14}-labeled piperonyl butoxide, α-[2-(2-n-butoxyethoxy)-ethoxy]-4,5-methylenedioxy-2-propyltoluene-α-C^{14}, by reacting dihydrosafrole with paraformaldehyde-C^{14} and condensing the chloromethyl product with sodium diethylene glycol monobutyl ether. The radioactive piperonyl butoxide was purified by vacuum distillation; the over-all yield was 61 per cent, and the specific activity was 0.157 $\mu c./mg$. Ultraviolet and infrared spectra were used to characterize the purity of the radioactive product.

FUMIGANTS

Winteringham & Hellyer (61) compared the effects of methyl bromide, ethylene dibromide, and ethylene dichloride on P^{32}-labeled intermediates extracted from the thoracic muscle of normal and poisoned adult house flies. The slow depletion of phosphoglycerate by the first two chemicals suggested a common inhibition of triose phosphate dehydrogenase, and thus they resemble iodoacetate in their action. Depletion of ATP and arginine phosphoric acid by methyl bromide indicated a rapid blocking of the phosphorylation of nucleotide acceptors.

ARSENICALS

Forgash (62) has concluded that male, adult American cockroaches are more susceptible than females to poisoning from injected solutions of arsenic trioxide. Last-instar nymphs of both sexes exhibit similar degrees of tolerance and are less susceptible than the adults. These differences in arsenic tolerance could not be explained on the basis of gross differences in glutathione content.

Out of a total of 39 organic arsenical compounds tested by Early & Cochran (63) in the laboratory, the most toxic were as follows: arseno-

methane As-1,2 disulfide, camphorated arsenomethane As-1,2 disulfide, and arsonated toxaphene. Boll weevils, rice weevils, southern army worm larvae, and cotton leafworm larvae were used as test insects. Arsonated toxaphene possessed the highest degree of toxicity and was more effective than toxaphene against the boll weevil. Phytotoxicity tests revealed that arsenomethane As-1,2 disulfide and its chlorinated derivative were about as toxic to cotton foliage as calcium arsenate.

The Toxic Action of Miscellaneous Chemicals

Kenaga (64) has evaluated the relationship of molecular weight to insecticidal activity employing a heterogeneous group of insecticides. Those compounds having the best insecticidal properties appear to have a molecular weight that most frequently falls between 240 and 414. The insecticides with the lower molecular weights appear to act best through contact and those with the higher molecular weights through ingestion.

Bettini & Boccacci (65) have observed that triosephosphate dehydrogenase (TPD) is inhibited *in vitro* and *in vivo* by chloroacetic and iodoacetic acids in *P. americana* and *M. domestica*. In their *in vitro* experiments, iodoacetic acid at concentrations of 0.0005 M inhibited 80 per cent of TPD. A parallel trend was observed in both the per cent TPD inhibition and kill in the *in vivo* experiments. After exposing adult house flies for 20 generations to chloroacetic acid, no increase of TPD in the *in toto* extracts was observed, suggesting that resistance towards this compound was not induced in this number of generations.

Weiden (66) attempted to demonstrate the *in vivo* acetylative conjugation of sulfanilamide in adult American roaches. Since negative results were obtained, several possible complicating factors were studied, with particular emphasis on the enzymatic deacetylation of N-acetylated arylamines. Acylase activity (hydrolysis of the amide linkage) against several compounds was demonstrated *in vitro* by tissues of the American cockroach and several other species. This enzyme appears to be widely distributed within the insect body, is not affected by dialysis, is rapidly inactivated by heating and its pH maximum is approximately at neutrality for phosphate-citrate buffers. It is also inhibited by a number of chemicals including several insecticides.

Although the conjugation of phenols with sulfuric acid in mammals is well known, less information is available concerning its occurrence in other organisms. Smith (67), using locusts and several other insect species, has shown that phenols injected into locusts are conjugated to a small extent to give compounds in the feces identical in electrophoretic and chromatographic properties with the corresponding ethereal sulfates. The latter metabolites are less toxic than the phenols to locusts. All the other insect species studied excreted phenols as β-glucosides, and in some cases ethereal sulfates were also present.

Naidu (68) has studied certain drugs and insecticides with reference to their site and mode of action not only to clarify conflicting views on the presence of cholinergic and adrenergic systems in insects, but also to investigate the physiological action of certain insecticides. The frequency of the beat of the isolated American cockroach heart, immersed in an aerated physiological solution, was used to evaluate the action of the following chemicals: acetylcholine chloride, atropine sulfate, nicotine, hexamethonium iodide, epinephrine (adrenaline), nor-adrenaline hydrochloride, ergotamine, eserine, pyrethrum, rotenone, pure parathion, para-oxon, and *p,p'* DDT. Antagonistic effects between some of these chemicals were also discovered.

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CHEMISTRY AND MODE OF ACTION OF ORGANOPHOSPHORUS INSECTICIDES[1,2,3]

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The chemistry and biochemistry of the organophosphorus insecticides are so closely interwoven that it seemed reasonable to discuss these aspects in the same section. An exhaustive bibliography is contained in recent reviews of organophosphorus insecticides by Casida (1) and systemic insecticides by Spindler (2); our discussion will be largely concerned with the chemistry and biochemistry of a few of these compounds with the introduction of some new and sometimes controversial material.

To be effective insecticides, organophosphorus compounds have rather narrow restrictions on their properties: there are limits on the solubility, molecular size, and stability, and if relatively stable they should be susceptible to conversion to an active material. Nevertheless, they range in stability from a half life at pH 8 and 25°C. of approximately 6 hr. to almost 100 years. Some are completely miscible with water, others have limited solubilities. Some react as such while many others are activated in the organism and by different mechanisms. Finally, some are contact poisons while others are systemic in their action.

Hartley (3) suggested that the prerequisites for systemic activity are water solubility, relative stability, and the capacity to be converted to a more active compound. Newer additions to this group have required modification of these prerequisites. For example, demeton has rather low water solubility, while Shell OS 2046 (dimethyl 1-carbomethoxy-1-propene-2-yl phosphate), although rather unstable, exhibits systemic activity and is not converted to a more active intermediate. Pyrazoxon [diethyl 3-methyl-pyrazolyl-(5)phosphorothioate] and Diazinon (diethyl 2-isopropyl-4-methyl-pyrimidyl phosphorothioate) do not differ radically in structure, yet only the former exhibits systemic action. It therefore seems that with our present knowledge we are not able to predict with certainty the requirements for systemic activity.

The aim of lower mammalian toxicity without appreciably reduced insecticidal effectiveness has been achieved to a considerable extent with compounds like malathion, Dipterex, and chlorthion. Possible explanations for low mammalian toxicities of malathion and Dipterex have been postulated

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[1] The survey of the literature pertaining to this review was completed in June, 1956.

[2] The following abbreviations are used in this chapter: ACh (acetylcholine); DFP (diisopropylfluorophosphate).

[3] Contribution No. 81, Science Service Laboratory, Canada Department of Agriculture, University Sub Post Office, London, Ontario, Canada.

as discussed below. In general, however, our knowledge is so limited that we are not able to explain adequately this differential toxicity nor to predict the structural requirements for it.

The Importance of the Cholinesterase System

The widely accepted view that the organophosphorus compounds kill insects by inhibiting their cholinesterase depends upon (*a*) the correlation between mortality and degree of *in vivo* cholinesterase inhibition for varying doses of insecticide [Chadwick & Hill (4)]; (*b*) the correlation between toxicity and *in vitro* anticholinesterase activity [Metcalf & March (5)]; (*c*) analogy with organophosphorus poisoning of mammals, in which class a large amount of work indicates that cholinesterase inhibition is the major lethal factor [Koppanyi (6); Nachmansohn (7); De Candole *et al.* (8)]; and (*d*) the failure to propose any other system which is effected at appropriately low concentrations and is of known importance to the insect.

This view has, however, been challenged on the grounds that insect phenylesterase may be inhibited by organophosphates and that ACh (acetylcholine), although toxic to mammals, is totally without effect when injected into insects.

Importance of other enzymes.—The background to this problem has been reviewed by Kearns (9). Lord & Potter (10) have shown the presence in certain insect preparations of an enzyme hydrolyzing phenyl acetate and not ACh. This enzyme is inhibited by organophosphates, and the problem is to decide how important is its inhibition in poisoning by these compounds.

O'Brien (11) has calculated that in the adult roach 50 per cent or less inhibition of phenyl esterase would be given by a lethal dose of tetraethyl-pyrophosphate (TEPP). Working with four other organophosphates Casida (12) concluded:

> The suggested implication of a phenylesterase in the primary mechanism of organophosphate poisoning of insects . . . does not appear to be applicable for the adult American cockroach, since the ACh esterase of the nerve was the only esterase consistently inhibited over 67% *in vivo* in cockroaches at the prostrate stage of poisoning . . . .

Neither Lord & Potter nor Hopf seem to have studied *in vivo* inhibitions of phenyl esterase in organophosphate poisoning. With cholinesterase of mammalian nerve, 90 per cent inhibition is required to abolish conduction [Nachmansohn (13)]. Up to 80 per cent inhibition of cholinesterase is required to kill roaches by TEPP, diisopropylfluorophosphate (DFP), or physostigmine (4). If similar degrees of inhibition are required before phenyl esterase inhibition is critical, such conditions are probably not reached at the LD_{50} dose.

Staudenmayer has shown that *Bombyx mori* (Linnaeus) eggs treated at various times with parathion are all killed two days before the normal hatching time (14); since cholinesterase develops five days before hatching (15) this suggests that death is unrelated to the enzyme inhibition. Smith (16) has reported similar results with eggs of the peach tree borer. The delayed

effectiveness of the insecticide suggests that it acts on some system un-essential to the egg but important to the larva or its immediate precursor. Van der Kloot (17), studying the pupating *Cecropia* silkworm, noted a lag of about two to three days between appearance of cholinesterase and of elec-trical activity in the brain. This is not altogether surprising, since in normally functioning tissue, cholinesterase is present in great excess, and presumably the system has to be completely "assembled" before activity begins. Should a similar lag be present in the *Bombyx* egg, onset of poisoning from para-thion would coincide with onset of electrical activity in the nervous tissue. Staudenmayer's figures (15) show that two days before hatching the rate of cholinesterase appearance starts levelling off, suggesting that the system is almost completed at this stage. If this is the true state of affairs, clearly the ovicidal action of parathion is attributable in the first instance to choline-sterase inhibition, but the inhibition is unimportant until the enzyme is needed for nervous activity.

This picture of the ovicidal effect of organophosphates fits the findings of Lord & Potter (18) for TEPP (which, being unstable, is ineffective unless the susceptible system is present at the time of application). Eggs of *Diataraxia* and *Ephestia* were only killed by very high concentrations of the insecticide, although they contained the phenyl esterase (an interesting period of in-creased sensitivity was noted within 24 hr. after oviposition).

The presence and importance of acetylcholine.—The evidence reviewed by Kearns (9, p. 143) clearly shows that ACh is present in insect tissues and that extracts of nervous tissue also contain a pharmacologically active com-pound. Is ACh the active compound? Fernando (19) concluded that the frog rectus- and *Venus* heart-positive material from roach nervous tissue was neither ACh nor a related compound, since it did not give a positive Hestrin test. However, in his recovery experiment, ACh added at over twice the level to be expected (if all the pharmacologically active compound in nerve were ACh) gave only a 6 per cent increase in optical density in the Hestrin test. Also his estimate of the expected level was probably high, since some of the pharmacological activity he observed was probably a result of trichloracetic acid remaining from the initial extraction (Kearns, private communication).

Chang & Kearns (20) have now shown that the only frog rectus-positive compound in roach nervous tissue after "homogenization, centrifugation, and extraction with acidified ethanol" had the same R_F as ACh, and gave a positive Hestrin test. This is fairly conclusive evidence that ACh is the only pharmacologically active compound present. Furthermore, the work of Smallman (21) on blowfly heads, by showing that synthesis of frog rectus-positive material depends upon a supply of choline, coenzyme A, and an acetyl donor, clearly indicates that large amounts of ACh can be produced by this preparation and that there is no other rectus-positive substance present in significant quantity. In this study frog rectus and guinea-pig ileum assays gave effectively the same estimate of the ACh level, making it unlikely that any other active compound was present.

Although ACh and its analogues are toxic to mammals (22), it has been shown by Tobias *et al.* (23) and Hopf (22) to be nontoxic to insects, and by Roeder (24) to be ineffective against synaptic transmission in the roach. On this basis, Hopf (22) has said: "We conclude, therefore, that it is doubtful whether insecticides do act as cholinesterase inhibitors." However, O'Brien (11), by studying the hydrolysis of various substrates by a minimally damaged insect, has shown that in the roach there is a barrier between ionized esters and their esterases. In support of this finding it was shown that the toxicity of a series of nitrogenous bases whose action in mammals is related to nerve transmission [e.g., epinephrine (adrenaline), nicotine, pilocarpine] showed toxicity ratios, insects: mammals, which were related to their ionization *in vivo* (as calculated from their pK's) in accordance with the hypothesis that only the unionized fraction penetrates to the site of action. The barrier responsible may be identical with that shown for potassium ions by Hoyle (25). The finding accounts for the nontoxicity of injected quaternary nitrogen compounds such as ACh and other choline esters, and the failure of atropine to relieve organophosphate poisoning in insects.

In summary, then, cholinesterase still seems the enzyme most significantly involved in most organophosphate poisoning, and ACh is probably the pharmacologically active component found abundantly in insect nerve (26).

Effect of organophosphates on nonesteratic sites.—One of the most important factors favouring the view that organophosphates are effective because of their anticholinesterase activity is the extensive evidence that this is the case in mammals. Recently Robinson *et al.* (27) and McNamara *et al.* (28) have observed a lack of correspondence between functional failure and cholinesterase inhibition in nerve-muscle preparations treated with DFP or TEPP. It was suggested that other factors than cholinesterase inhibition were involved. The conclusion is probably correct, although it is difficult to rule out the possibility that the cholinesterase assayed was total enzyme, while the critical fraction might by very much less and its fluctuations might not be a simple reflection of those observed in the total.

Convincing evidence has been produced by Cohen & Posthumus (29) that the effect of DFP and Sarin on the frog rectus involves factors additional to cholinesterase inhibition. They suggest that another type of inhibitable group also exists in muscle which modifies the response of the muscle to effectors. Cohen *et al.* (30) suggest that these sites are structurally similar to anionic sites of cholinesterases. Similar studies on the response of a phrenic nerve-diaphragm preparation to DFP led Van der Meer & Meeter (31) to propose that some of the effects were the result of factors other than cholinesterase inhibition. Roeder & Kennedy (32), working with the last abdominal ganglion of the roach, also found cholinesterase inhibition inadequate to explain the effects of certain phosphinates. They propose that these compounds (and possibly DFP and TEPP) also act directly upon nerve receptors.

Although malathion is a moderate anticholinesterase, it is converted to

a stronger one by insects [Metcalf & March (33)]. O'Brien (34), studying the effect of malathion on flies and roaches, found that it produced a marked inhibition of the cholinesterase of poisoned flies initially. However, by the time death occurs the cholinesterase is largely reactivated, and therefore inhibition of this enzyme may not be the cause of death. Unfortunately there is a lack of detailed evidence on the time-course of cholinesterase inhibition in poisoning of insects by "typical" anticholinesterases. The respiratory pattern following malathion poisoning was also unlike that of other organophosphates. Certainly malathion has little effect on mammalian carbohydrate metabolism *in vitro* [Hosein *et al.* (35)].

DETOXIFICATION AND ACTIVATION

Detoxification.—Clearly the toxicity of an organophosphorus compound to a species will be profoundly effected by the presence of enzymes capable of splitting the compound to inactive products. In mammalian tissues the presence has been shown of enzymes capable of hydrolyzing DFP [Augustinsson & Heimburger (36); Mazur (37); Mounter *et al.* (38)] TEPP [Mounter (39)], and "paraoxon" [Aldridge (40)]. The important influence of such enzymes upon the toxicity of their substrates to mammals has been shown by Saunders (41) and Adie (42). In insects Metcalf *et al.* (43) have found an aromatic esterase capable of hydrolyzing parathion and "paraoxon." There is evidence against the existence of insect enzymes hydrolyzing schradan or its active metabolite [O'Brien & Spencer (44)].

The degradation of Dipterex in the mammal is very rapid, and this may account for its low mammalian toxicity [DuBois & Cotter (45)]. Arthur & Casida (46) suggest that in both mammals and insects the breakdown occurs by direct cleavage of the P—C bond without prior rearrangment.

Malathion breakdown in the hen, mouse, and roach has been studied by March *et al.* (47). The mouse and hen (to both of which malathion is almost nontoxic) appear to degrade the insecticide through similar intermediates, involving hydrolysis, in two stages, of the diethyl succinate nucleus, and hydrolysis of the P—S—C link (it is uncertain whether the cleavage is at the P—S or the S—C bond). Each of these alterations can occur with or without prior oxidation of the P→S. There is evidence that all of the seven possible intermediates with intact P—S—C bonds are in fact produced. These intermediates make up the bulk of the compounds eliminated during the early, rapid phase of excretion. This extensive metabolism, as well as the rapid excretion, may well account for the low toxicity of malathion to these animals. Malathion metabolism was also studied in the roach, to which the compound is toxic. In contrast with the mouse and hen, metabolism is not extensive, as judged by the limited number of products: chromatography of the metabolic products showed only two spots. One of these was probably given by malathion and the analogous P→O compound [*O,O*-dimethyl S-(1,2-bis-carboethoxy)-ethyl phosphorothiolate], which were not resolved.

Demeton (Systox) degradation in the mouse and roach was studied by

March *et al.* (48). Two important pathways are (*a*) the oxidation of the thiono isomer and its sulfoxide and sulfone metabolites to the phosphates, which are then susceptible to nonenzymic hydrolysis, (*b*) enzymatic hydrolysis of the P—O- or P—S-ester links to produce the nontoxic acids and alcohols. Both routes seem to occur in both animals, but in general the rates are greater in the mouse.

In those cases where there exist detoxifying enzymes of such activity as to exert a marked influence on the toxicity of a compound, one might expect to find a toxicity lower for mammals than for insects (other factors being equal) simply by virtue of the difference in body temperature. However, enzymatic reaction rates commonly vary by a factor of about 2 for a 10° C. temperature change [Stearn (49)], and this is quite insufficient to account for the differential toxicity of, say, malathion or Dipterex.

Differential degradation rates could be exploited in designing insecticides of low mammalian toxicity. The presence in mammals, and not in insects, of an esterase, for instance, of high specificity with respect to a particular linkage irrespective of the nucleus to which it was attached, would mean that the introduction of the ester grouping into a toxic nucleus would assure the hydrolysis of the ester link in mammals. This could be arranged to lower the toxicity of the compound, either by altering its penetrability because of the resultant ionization (11, 50, 51) or by the modification of the character of neighbouring groups produced by substituting the nucleophilic carboxylate ion for an electrophilic alkoxyl group.

Activating systems.—Compounds such as schradan, parathion, and malathion are weak anticholinesterases but are converted to powerful inhibitors ("activated") by certain enzyme systems. The conversion of several thiophosphates to the phosphates has been shown in roach gut by Metcalf & March (52), and parathion activation in roach fat body has been shown by Kok & Walop (53).

O'Brien & Spencer (44, 54) have shown that, in several tissues from all insects studied, schradan is activated by conversion to a compound which they now believe to be the methylol derivative (55). Heath *et al.* (56), using oxygenated liver slices, showed that two powerful anticholinesterases are produced from schradan, as well as the weaker inhibitor heptamethyl-pyrophosphoramide. The study of activation in mammals has been greatly facilitated by the work of Davison (57), who obtained a microsome-plus-soluble preparation from rat liver which, when fortified with diphospho-pyridine nucleotide, nicotinamide, and magnesium, converted both schradan and parathion to powerful anticholinesterases. He demonstrated a difference in the distribution of the activating systems for these two compounds between the sexes. Two different enzymes are therefore involved, presumably a thionophosphate oxidase and an amide oxidase. The schradan activating system was further studied by O'Brien (58) who found it present in the livers of all of the eight mammals studied and also in several nonhepatic tissues. A peroxidase mediated system was proposed, for which further evi-

dence has now been provided (59). The insect conversion system is different from the mammalian one in being inhibited by phosphate and not fortified by the same cofactors [O'Brien (59)].

Demeton (Systox) activation in the mouse and roach was studied by March *et al.* (60). Both isomers may be oxidized, at the ethylmercaptoethyl sulphur, first to the sulfoxide then to the sulfone. In contrast to parathion, in which the oxidation of the thiono sulphur to give a phosphate is of great importance for toxicity and anticholinesterase activity [Gage (61); Metcalf & March (52)], the analogous oxidation of the thiono isomer of demeton is primarily a detoxifying step, since the resulting phosphate is very unstable. The problem is a complex one and is dealt with more fully below, in the discussion on demeton.

The attractive idea that DDT-resistant flies, having a highly active enzyme capable of dehydrochlorinating DDT, might have a similar effect on Dipterex [Babers & Mitlin (62)] has not been fulfilled. Instead, Arthur & Casida (46) suggest that direct phosphonate link cleavage occurs. Nonenzymic dehydrocholorination produces the highly active DDVP (dimethyl 2,2-dichlorovinyl phosphate) [Mattson, Spillane & Pearce (63)] which is 10 times as toxic to flies as Dipterex itself and a more powerful anticholinesterase. Arthur & Casida (46) have also demonstrated another interesting activation process: the enzymatic hydrolysis in the rat and various insects of 0,0-dimethyl 2,2,2-trichloro-1-acetoxyethyl phosphonate to its deacetylated form, Dipterex.

SELECTIVITY BETWEEN INSECT SPECIES

Indiscriminate insecticides are seldom desirable. Rapid resurgence of the pest population commonly follows their use [Ripper (64)] and the long term effect upon the complexly interrelated balance of species is unpredictable and may be disastrous. Selectivity can be obtained (*a*) by taking advantage of ecological or behavioural habits, as in the use of systemic insecticides which are taken up only by phytophagous insects; and (*b*) by using insecticides whose intrinsic toxicity differs with different species. The latter aspect will be discussed here.

There are close similarities in the insecticidal specificity of three important insecticides: schradan, whose selective aphicidal activity has long been known, and two new compounds, one the methosulfate of the thiol component of demeton (I), the other 0,0-diethyl-S-β-diethylaminoethyl phosphorothiolate (II).

$$
\begin{array}{c}
C_2H_5-O \quad\quad O \\
\diagdown\, P \diagup \\
C_2H_5-O \quad S-CH_2-CH_2-\underset{\underset{+}{|}}{S}-CH_2CH_3 \\
CH_3 \\
[CH_3SO_4]^-
\end{array}
$$ I

$$C_2H_5-O \quad O$$
$$\diagdown \; \nearrow$$
$$P$$
$$\diagup \quad \diagdown$$
$$C_2H_5-O \quad S-CH_2-CH_2-N \diagdown \begin{matrix} C_2H_5 \\ \\ C_2H_5 \end{matrix}$$

II

Fukuto *et al.* have synthesized (I) (65), and (II) was synthesized by Ghosh & Newman (66). It is from these authors that the data on (I) and (II) are drawn. The data on schradan are from Ripper *et al.* (67), de Pietri-Tonelli & March (68), and O'Brien & Spencer (54).

All three compounds are effective anticholinesterases (although schradan requires activation first), toxic to mammals, and effective against aphids, spider mites, and mosquito larvae. Unfortunately the data on resistant species do not overlap, but (I) is nontoxic to house flies, (II) to lady beetles and flour beetles, schradan to house flies, mealworm larvae, Colorado potato beetles, roaches, and honey bees. The pattern is then suggestively similar, and further work may show an identity.

(I) and (II) have a similarity even more important than the common triethyl phosphorothiolate nucleus, in that both should be almost totally ionized at all physiological pH's: (I), being quaternary, is ionized at all pH's, and in (II) the nitrogen should have a pK_a not substantially different from that of triethylamine (10.8) from which it may be calculated that at pH 7, over 99 per cent is ionized. Now this is very unusual in insecticides. Introduction of a quaternary group into carbamate insecticides eliminates insecticidal activity towards the house fly in spite of improving the *in vitro* anticholinesterase activity [Kolbezen *et al.* (51)]. Insecticides with alkyl-substituted nitrogen are usually amides (schradan, carbamates) or have the nitrogen in an unsaturated ring (Diazinon, Pirazinon, Isolan, Pyrolan); such nitrogens have low pK_a's [Brown *et al.* (69)] and are not normally ionized. The toxicity to the German roach of several neuropharmacological nitrogenous bases has been shown by O'Brien (11) to be inversely related to the alkaline range pK_a. The limited data available therefore suggest that the introduction of an ionizable group produces a selective toxicity, suscept-ible insects being aphids, spider mites, and mosquito larvae. The results on carbamates (51) and (I) (65) both suggest that thrips occupy an inter-mediate position. Mammalian toxicity is not reduced and may be enhanced.

The schradan susceptibility picture is similar to that of (I) and (II), but neither schradan nor its anticholinesterase metabolite are normally ionized. Although the active anticholinesterase metabolite is more hydrophilic than the parent compound, it is not strikingly polar: its chloroform-water parti-tion coefficient is 1.7 [O'Brien & Spencer (44)]. Furthermore, heptamethyl-pyrophosphoramide, which has a very similar partition coefficient, displays the same toxicity pattern as schradan [Spencer *et al.* (55)], and therefore it or its metabolites (which would almost certainly be more polar) can pene-trate to the site of action. Considerations of polarity thus give no grounds for the hypothesis that in the German roach or squash bug schradan can pene-

trate to the site of action but its anticholinesterase metabolite cannot. The hypothesis had been used by O'Brien & Spencer (54) in an attempt to account for the nontoxicity of schradan to the roach, for they observed that the roach and other nonsusceptible species had an even greater capacity than susceptible insects to convert schradan to an active anticholinesterase metabolite by nonnervous tissue. They proposed that the metabolite could not penetrate to the active site, and therefore only the schradan which escaped conversion by nonnervous tissue was effective in poisoning. As all insects studied have been shown to convert large quantities of schradan to the active anticholinesterase metabolite (54), it is an attractive hypothesis that the resistant insects have a barrier preventing entry of the metabolite to the site.

SCHRADAN (OCTAMETHYLPYROPHOSPHORAMIDE)

Although schradan (III) was one of the first systemic insecticides to be developed, its mode of action is still a subject of controversy and all the products of chemical oxidation have still not been identified. Hartley (3) was the first to postulate that this activation possibly consisted of an oxidation to a phosphoramide N-oxide (IV) with several alternative subsequent pathways including a rearrangement to a methylol derivative (V) and subsequent splitting out of formaldehyde, leaving heptamethylpyrophosphoramide (VI).

$$RO-P(O)N(CH_3)_2 \qquad RO-P(O)-N(O)(CH_3)_2 \rightarrow$$
$$\underset{N(CH_3)_2}{|} \xrightarrow{\text{oxidation}} \underset{N(CH_3)_2}{|} \qquad \rightarrow$$
$$\text{III} \qquad\qquad\qquad \text{IV}$$

$$RO-P(O)-N-CH_2OH \qquad RO-P(O)-NH + CH_2O$$
$$\underset{|}{|}\quad CH_3 \longrightarrow \underset{|}{|}\quad CH_3$$
$$N(CH_3)_2 \qquad\qquad N(CH_3)_2$$
$$\text{V} \qquad\qquad\qquad \text{VI}$$

or
$$RO-P(O)-N-OCH_3$$
$$\underset{|}{|}\quad CH_3$$
$$N(CH_3)_2 \qquad \text{where R} \quad [(CH_3)_2N]_2P(O)-$$
$$\text{VII}$$
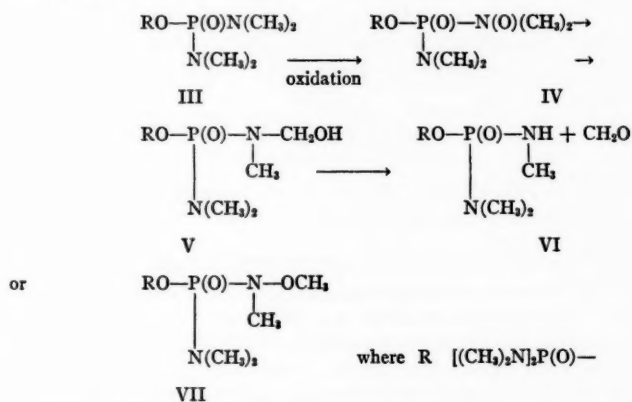
The major active anticholinesterase obtained by permanganate oxidation of schradan has been shown to be identical with that produced metabolically by mammals and plants [Casida *et al.* (70, 71)] and insects [O'Brien & Spencer (44)]. The latter suggested that this active anticholinesterase, which is over 100,000 times as active as schradan itself as a cholinesterase inhibitor. might be the amide oxide (IV) or the methylol derivative (V) of schradan. In a recent paper (72) Tsuyuki *et al.* (a) suggest that the active anticholinesterase is the amide oxide (IV), primarily on the basis of

the 1681 cm.$^{-1}$ peak found in schradan oxidation mixtures, which they compare with the 1675 peak present in trimethylamine oxide dihydrate and lacking in trimethylamine; (*b*) show that under acid or alkaline conditions, or on heating, the active anticholinesterase isomerizes to a much more stable component which they suggest is the methyl ether (VII). Heath *et al.* (56), however, show that the partition coefficient of the active component fits the methylol (V) rather than the amide oxide (IV) structure. Spencer *et al.* (55, 73) have shown that (*a*) the peak in the 1690 cm.$^{-1}$ region remains after the active anticholinesterase is removed by mild hydrolysis and is only eliminated by hydrolysis under severe conditions; (*b*) the active anticholinesterase and the compound with a 1690 cm.$^{-1}$ absorption occur in different fractions in countercurrent analysis; (*c*) that the only peak between 1600 and 1700 cm.$^{-1}$ present in trimethylamine oxide dihydrate disappears upon dehydration and is therefore not attributable to the N→O link, and (*d*) that the methyl ether (VII), which they synthesized, was not present in the schradan oxidation mixture, as evidenced by countercurrent distribution studies.

Spencer *et al.* (55, 73) therefore suggest that in the metabolic or chemical oxidation of schradan the amide oxide structure is probably only a transitory intermediate and agree with Heath *et al.* (56) that the active anticholinesterase is probably the methylol derivative (V). Further evidence, by analogy, favouring the methylol structure is given by Fish *et al.* (74) as well as others cited in reference (55). The active intermediate either hydrolyzes or phosphorylates, or under certain conditions splits out formaldehyde, yielding the much more stable heptamethylpyrophosphoramide. The material with an infrared absorption in the 1690 cm.$^{-1}$ region may be the carboxyl derivative

$$-P-N-\overset{\displaystyle H}{\underset{\displaystyle |}{C}}=O$$
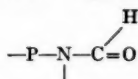
mentioned in 1951 by Hartley (3), but it is not the active anticholinesterase and probably not the phosphoramide N-oxide.

Demeton and Other Alkyl Monothiophosphates

Although the systemic organophosphorus insecticide demeton was introduced more recently than schradan, the chemistry of its metabolic products has been more completely elucidated in spite of added complications of sulfur isomerization. Initially this compound appeared unusual because it was both a systemic and a contact poison and, unlike schradan, had a very low water solubility. Gardner & Heath (75) showed that the commercial product was composed of two isomers, *O,O*-diethyl *O*-ethylthioethyl phosphororothionate and a thiol isomer *O,O*-diethyl *S*-ethylthioethyl phosphorothiolate. The latter, although only slightly water soluble, is about 10 times more so than the former, according to Schrader (76). The thiol isomer is rather more toxic than the thiono to the mouse (76), in contrast to thiol isomers of parathion-type insecticides, which are less toxic than their parent compounds (77). The anticholinesterase activity of the thiol is almost

a hundredfold greater than that of the thiono isomer as shown by Fukuto *et al.* (78). Similar physical properties and the differential activity were reported by Von Rümker (79).

Isomerization occurs from the thiono to the thiol isomer. Whereas in parathion the isomerization takes place between the thiono sulfur and the ethoxy oxygen, in demeton the mercaptoethoxy oxygen is involved. Metcalf & March (77) showed that the former and its homologues require a relatively high temperature (150°C.) while Fukuto & Metcalf (80) found that isomerzation of the latter is fairly extensive at 37°C. in an appropriate polar solvent. They also showed that demeton isomerization follows first order kinetics and is enhanced by polar solvents suggesting that of the two mechanisms proposed, that involving the ionic intermediate is more probable, as shown in the following reaction:

$$
\begin{array}{ccc}
\mathrm{C_2H_5-O} & \mathrm{S} & \\
& \diagdown\mskip-5mu\nearrow\!\!\mathrm{P} & \mathrm{S-C_2H_5} \\
\mathrm{C_2H_5-O} & \mathrm{O-CH_2CH_2} &
\end{array}
\qquad
\begin{array}{ccc}
\mathrm{C_2H_5O} & \mathrm{S-CH_2CH_2} & \\
& \diagdown\mskip-5mu\nearrow\!\!\mathrm{P} & \mathrm{S-C_2H_5} \\
\mathrm{C_2H_5O} & \mathrm{O} &
\end{array}
$$

$$
\left[\begin{array}{cc}
\mathrm{C_2H_5-O} & \mathrm{S} \\
& \mathrm{P} \\
\mathrm{C_2H_5-O} & \mathrm{O^-}
\end{array}\right]
\left[\begin{array}{c}
\mathrm{CH_2} \\
\quad \mathrm{S^+-C_2H_5} \\
\mathrm{CH_2}
\end{array}\right]
\ \rightleftarrows\
\left[\begin{array}{cc}
\mathrm{C_2H_5O} & \mathrm{S^-} \\
& \mathrm{P} \\
\mathrm{C_2H_5O} & \mathrm{O}
\end{array}\right]
\left[\begin{array}{c}
\mathrm{CH_2} \\
\quad \mathrm{S^+-C_2H_5} \\
\mathrm{CH_2}
\end{array}\right]
$$
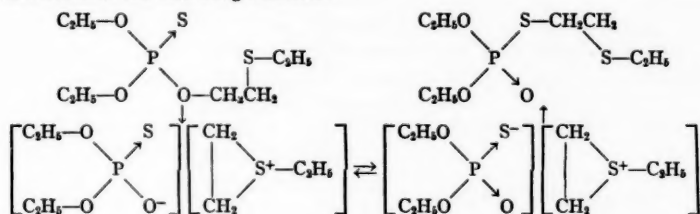
Support for this mechanism was found by Henglein & Schrader (81) in their study of the isomerization of both demeton and the dimethyl ester (Metasystox), on the basis of the polar solvent effect and the absence of a free radical mechanism, as tested by acrylonitrile polymerization. Furthermore the sulfone derivative of the thiono isomer did not isomerize, which is understandable in the light of the above mechanism. One would predict that those thiono-phosphates containing the alkylthiomethyl moiety would not readily isomerize because the sulfonium ring structure could not be formed.

By analogy with the oxidation of the thiono sulfur of parathion one might expect the more stable thiono derivative of systox to be oxidized to the corresponding phosphate. However, the problem is complicated by the presence of the mercapto sulfur which can be oxidized to the sulfoxide and finally to the sulfone; from the thiono and thiol isomers a total of seven oxidation products are possible. The metabolism in the mouse and cockroach has been examined extensively by March *et al.* (48) and in plants by Metcalf *et al.* (49). The results seem to indicate that by contrast with parathion, the oxidation of the thiono sulfur is of secondary importance and that the oxidation of the mercapto sulfur the sulfoxide and possibly to the sulfone predominates. Heath *et al.* (56) have demonstrated the existence of two products from thiolate oxidation by the plant. Table I shows data from Fukuto *et al.* (78) for fly brain cholinesterase inhibition which have been calculated for the thiono and thiol isomers of demeton and the seven oxidation products. Data on stability to hydrolysis are also given.

Aldridge (83) showed a high correlation between hydrolyzability and

TABLE I

FLY BRAIN CHOLINESTERASE INHIBITION AND HYDROLYSIS OF THE OXIDATION
PRODUCTS OF DEMETON*

Compound	Fly-brain cholinesterase inhibition pI 50†	Mole per cent hydrolysis at pH 7.9, 37°C., 60 min.
1. $(C_2H_5O)_2\overset{\textstyle S}{\overset{\uparrow}{P}}OC_2H_4SC_2H_5$	3.7	9.4
2. $(C_2H_5O)_2\overset{\textstyle O}{\overset{\uparrow}{P}}SC_2H_4SC_2H_5$	5.5	19.6
3. $(C_2H_5O)_2\overset{\textstyle O}{\overset{\uparrow}{P}}OC_2H_4SC_2H_5$	7.6	39
4. $(C_2H_5O)_2\overset{\textstyle S}{\overset{\uparrow}{P}}OC_2H_4\overset{\textstyle O}{\overset{\uparrow}{S}}C_2H_5$	5.5	6.2
5. $(C_2H_5O)_2\overset{\textstyle O}{\overset{\uparrow}{P}}SC_2H_4\overset{\textstyle O}{\overset{\uparrow}{S}}C_2H_5$	5.8	1.6
6. $(C_2H_5O)_2\overset{\textstyle O}{\overset{\uparrow}{P}}OC_2H_4\overset{\textstyle O}{\overset{\uparrow}{S}}C_2H_5$	6.0	13.7
7. $(C_2H_5O)_2\overset{\textstyle O}{\overset{\uparrow}{P}}OC_2H_4\overset{\textstyle O}{\underset{\textstyle O}{\overset{\uparrow}{\underset{\downarrow}{S}}}}C_2H_5$	6.9	—
8. $(C_2H_5O)_2\overset{\textstyle O}{\overset{\uparrow}{P}}SC_2H_4\overset{\textstyle O}{\underset{\textstyle O}{\overset{\uparrow}{\underset{\downarrow}{S}}}}C_2H_5$	6.2	9.7
9. $(C_2H_5O)_2\overset{\textstyle S}{\overset{\uparrow}{P}}OC_2H_4\overset{\textstyle O}{\underset{\textstyle O}{\overset{\uparrow}{\underset{\downarrow}{S}}}}C_2H_5$	6.1	10.1

* From data of Fukuto *et al.* (78).
† Negative logarithm of molar concentration required to inhibit 50 per cent of cholinesterase activity.

anticholinesterase activity in a series of organophosphorus compounds. However, it should be noted that beyond a certain instability level the anticholinesterase activity declines, probably as a result of hydrolysis of the compound before it reaches the enzyme [Spencer & O'Brien (84)]. It is of interest to note in this connection that phosphate No. 3, Table I, has the

highest pI_{50} and is most readily hydrolyzed. The sulfoxide of phosphate No. 6 is more stable to hydrolysis and less active as an *in vitro* anticholinesterase, but the sulfoxide of the thiono isomer (No. 4) is 100 times more reactive than the thiono isomer itself (No. 1) as an anticholinesterase, although slightly more stable to hydrolysis. Further examination of the table will indicate other "irregularities," emphasizing the danger of generalizations and suggesting the interplay of several factors in determining activity.

Fukuto *et al.* (65) observed that formation of the sulfonium salt of the thiono isomer increased the anticholinesterase activity a hundred fold. A similarity in structure between the sulfonium salt and the natural cholinesterase substrate acetylcholine was noted. The quaternary aminophenyl, quinolyl, and pyridyl alkyl phosphates mentioned by Avison (85) contain a similar structure and are also powerful anticholinesterases.

A new insecticide has been reported by Ghosh & Newman (66): O,O-diethyl, O-diethylaminoethyl phosphorothionate. Besides having a thiono sulfur that isomerizes to the thiol form it also includes a basic nitrogen. Isomerization from the thiono to the thiol alkylamino isomer occurs and presumably follows a mechanism similar to that for demeton since there is the possibility of an onium ring structure:

$$\begin{bmatrix} C_2H_5O & S \\ & \nearrow \\ & P \\ C_2H_5O & O^- \end{bmatrix} \begin{bmatrix} CH_2 & C_2H_5 \\ & N^+ \\ CH_2 & C_2H_5 \end{bmatrix}$$
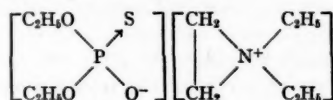
It will be noted that the spatial relation of the phosphorus and the nitrogen is similar to that of sulfonium salt of Fukuto *et al.* (65) and ACh. Therefore the similarity to the natural substrate of cholinesterase aids in the formation of the inhibitor-enzyme complex and adds to the activity of this compound, particularly to that of the thiol isomer. Perhaps because of the basic nitrogen and consequent salt formation, great differences in toxicities to different groups of insects and mites are shown. Thus good *in vitro* activity of a compound against a vital enzyme may be useless if the compound fails to penetrate to the enzyme *in vivo*. This material exhibits both contact and systemic insecticidal activity. It will be of interest to find whether, during metabolism, the nitrogen is oxidized to an amine oxide.

MALATHION AND OTHER DITHIOPHOSPHATES

A series of organophosphorus compounds somewhat similar to demeton are the O,O-dialkyl S-alkythiomethyl phosphorodithioates. They differ from demeton in two important ways: in having a mercaptomethyl group instead of a mercaptoethyl, and a dithioate instead of thioate structure. The dithioate structure precludes the possibility of the isomerization from the thiono to the thiol form found in demeton. Further work will indicate the relative importance of thiono sulfur and mercaptomethyl sufur oxidation. Preliminary investigation by Clark *et al.* (86) has shown the limitations in choice of alkyl radicals to give maximum systemic activity. One of the three most

active compounds is *O,O*-diethyl *S*-isopropylthiomethyl phosphorodithioate (Thimet). In addition to having systemic activity it is also a contact poison, and its toxicity is similar to parathion in being high to both mammals and insects.

Malathion (*O,O*-dimethyl *S*-1,2-dicarbethoxyethyl phosphorodithioate) differs radically from Thimet in that is toxicity for mammals is much less than for insects. An improved estimation of malathion by Jura (87) is based on the alkaline hydrolysis and polarographic estimation of fumaric acid, rather than the colorimetric estimation of the more unstable *O,O*-dimethyl phosphorodithioic acid of Averell *et al.* (88). O'Brien (34) has shown that the conditions and route of isomerization are similar to those for parathion.

Alkyl Aryl Thiophosphates

In the search for organophosphorus insecticides with lower mammalian toxicity interest has returned to chlorinated derivatives of parathion. Oddly enough both monochloro derivatives, the 2-chloro (Am. Cyanamid 4124) and the 3-chloro (chlorthion) have much lower mammalian toxicity than parathion and only slightly less insect toxicity. Schrader (89) has shown that substitution of various additional substituents in the ring reduces mammalian toxicity but also reduces insecticidal activity, rendering them useless for practical purposes. There is some ambiguity about the oral toxicity to rats of the 2-chloro compound: Schrader (89) gives 200 mg./kg. as the LD_{100}, while the American Cyanamid commercial release quotes 1125 mg./kg. as the acute oral toxicity.

The trichloro analogue, DOW ET-57 (*O,O*-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate) has extremely low mammalian toxicity (90) and has recently been shown by Lindquist (91) to be highly effective in the systemic control of grubs of the warble fly, *Hypoderma lineatum* (De Villiers). Doses of 100 mg./kg. administered orally gave good control and were apparently without adverse effect on the cattle. The compound was more effective than others previously tested in that it killed larvae before cyst formation in the back had occurred.

Dipterex and Other Alkyl Phosphonates

Except for *O*-ethyl *O-p*-nitrophenyl phenylphosphonothionate (EPN) most of the organophosphorus insecticides developed to date have been phosphates, phosphorothioates, or phosphorodithioates. Thus the introduction of the *O,O*-dialkyl 1-hydroxyethylphosphonates was of special interest. The general method for preparation was mentioned first by Craig & Hester (92) and later by Fields (93). It remained for Lorenz (94) specifically to mention in his patient application the dimethy lester, prepared by reacting chloral with the methyl phosphite to yield *O,O*-dimethyl 1-hydroxy-2-trichloroethylphosphonate (Dipterex). Barthel *et al.* (95) described the synthesis of a homologous series. The behaviour of Dipterex towards alkali was unexpected: following dehydrohalogenation the 1-keto-2,2-dichloroethylphosphonate might be expected. Instead, an unusual rearrangement followed,

yielding a vinyl phosphate. This rearrangment was reported almost simultaneously by Barthel *et al.* (96), Lorenz *et al.* (97), and Mattson *et al.* (98), these last authors naming it DDVP, for *O,O*-dimethyl 2,2-dichlorovinylphosphate. A mechanism for the rearrangement has been proposed by Kharasch & Bengelsdorf (98a).

The particular value of Dipterex is its relatively low mammalian toxicity yet good insecticidal properties. The possible mode of action and selectivity is discussed above.

VINYL PHOSPHATES

In the identification of the dehydrohalogenated and rearranged product of Dipterex, the reaction of a trialkylphosphite with a chlorinated aldehyde as outlined by Perkow *et al.* (99) was used. This general method has been studied further by Perkow *et al.* (100) and Allen & Johnson (101), and both have postulated mechanisms of reaction. The latter extended the investigation to include the reaction of *a*-halogen aldehydes, ketones, and esters and amides of certain carboxylic acids with completely esterified trivalent phosphorus. The final product was identified as the vinyl ester of the corresponding phosphoric acid.

Perkow *et al.* (99) mentioned that a number of these dialkyl chlorovinyl phosphates exhibited miotic effects. One of these compounds has been patented by Stiles (102) who reacted trimethyl phosphite with 2-chloro-aceto-acetate to yield *O,O*-dimethyl 1- carbomethoxy-1-propene-2-yl posphate (Shell OS 2046). It is of particular interest as a systemic insecticide with a short persistence in the plant, being dissipated in about two days [Casida *et al.* (103)]. This compound has two geometrical isomers, by contrast with the structural isomers of the monothiophosphates. A detailed study by Casida *et al.* (103) has shown that the *cis* isomer of compound OS 2046 is 100 times more toxic to insects and mammals than the *trans* isomer. The mechanism of action is further complicated by an initial enzymatic hydrolysis of the carboxylic ester group in the plant. This vinyl phosphate also has fumigant properties but of possibly greater significance is its type of systemic action: the *cis* form is a strong anticholinesterase and further activation does not occur. The large difference in biological activity between the *cis* and *trans* isomers emphasizes the importance of special configuration in specificity of action.

NOMENCLATURE

The nomenclature of organophosphorus compounds is in a confused state. In 1952, following much debate between the British and American workers, an internationally agreed compromise system of nomenclature was published for naming compounds containing one atom of phosphorus (104). Many major journals, including the publications of the American Chemical Society, have accepted the system, but the *Journal of Biological Chemistry* seems to have no intention of doing so. In the publications of the American Entomological Society it is not yet used in all papers.

Since common names are unfortunately not available for many of the

newer compounds, it is necessary to use proprietary names, which, however, show signs of being obscured in a maze of code numbers; for instance Dipterex should be called Bayer L 13/59 according to the American Entomological Society. In a supplement to the list of "Common" Names of Insecticides (105), 12 out of the 18 names consist of the name of a Company followed by a code number. The problem is further complicated by the use of different numbers for different formulations; thus DOW ET-57 is a purer form of DOW ET-14. Such code names are excellent advertisements but are unsuitable for scientific literature!

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THE BEHAVIOUR OF SYSTEMIC INSECTICIDES APPLIED TO PLANTS[1]

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In 1947 Martin (53) described insecticidal chemicals that are absorbed and translocated in plants as systemic, and, although other workers have suggested terms such as chemotherapeutic and teletoxic, systemic has become the most generally accepted. Bennett (6) defined a systemic insecticide as a substance which is absorbed and translocated to other parts of the plant, thus rendering untreated areas insecticidal. Unterstenhöfer (70) amplified this by including temporary accumulation as another important factor which governs the period of effectiveness of the preparation. There is a large number of nonionic organic insecticides in use today which penetrate the plant, and a list of such materials has been collected [Gunther & Blinn (37)]. As these materials are soluble in plant lipoids, penetration at least into the cuticle and often to the sub-cuticular layers might be expected; some are translocated but generally in insufficient quantities to be insecticidal and so do not qualify as systemic although a strong localized action may occur. More recent work indicates that borderline cases exist, as some of the insecticides claimed to be systemic show only limited translocation under some conditions, while others not considered systemic are translocated more than might be expected.

Whilst the term "systemic" is of recent origin the concept of such a method for pest and disease control dates back many centuries. The first systemic insecticides were found in plants, and the most fully explored chemical of this kind is sodium fluoroacetate which is found in the poisonous South African plant, *Dichapetalum cymosum*. David & Gardiner (21) showed that sodium fluoroacetate acted as a systemic insecticide in broad bean plants but suggested that its mammalian toxicity was too high for it to be of practical importance. Selenium was the first systemic insecticide to be studied closely by entomologists following the observations that wheat growing on seleniferous soils was not attacked by aphids [Hurd-Karrer & Poos (46)]. It has been demonstrated that there is a correlation between the rates of absorption of sulphur and selenium by different plants, and that the sulphur-selenium ratio of the plant tissue rises as the sulphur content of the seleniferous soil is increased [Hurd-Karrer (45)]. It would appear probable that selenium is able to replace sulphur in the metabolism of the plant, and it has been suggested that in some cases the insecticidal action is attributable to replacement of sulphur by selenium in some sulphur containing vitamins or proteins vitally concerned in reproduction [Bennett & Martin (7)].

Fulton & Mason (32) in 1937, produced the first evidence for the absorp-

[1] The survey of the literature pertaining to this review was completed in April, 1956.

tion and translocation of a bulky insecticidal molecule foreign to the plant, when they found that derris applied to the first two leaves of bean plants reduced the attack by the Mexican bean beetle on leaves subsequently produced.

The discovery by Schrader (63) of systemic insecticidal properties in widely separated types of compounds (the most effective being certain derivatives of fluoroethyl alcohol and certain alkylamino-phosphorous compounds) started and gave impetus to the large volume of work on systemic insecticides carried out over the last 10 years. The application of this new discovery to the field of plant protection has resulted in a large number of publications mostly referring to the results of field trials against specific pests. No attempt has been made in this review to include this section of the literature, but attention has been concentrated on plant physiology connected with the use of systemics. Readers interested in more complete bibliographies on the subject are referred to the works of Ripper (60), Giang (35), and Spindler (64).

Ripper (60) has classified materials possessing systemic behaviour into the three following groups according to the decomposition within the plant: (a) stable insecticides such as selenium where the toxicity is attributable to the selenate ion which is not decomposed; (b) endolytic insecticides such as schradan and dimefox, which remain in the plant in their original form and act as insecticides until decomposed by the plant; (c) endometatoxic insecticides such as demeton, which are metabolised partly or wholly in the plants into other toxicants.

The distinction between the last two groups is ill defined since the biochemical changes which these materials undergo in the plant are often very complex. Thus the presence of active cholinesterase inhibitory metabolites of schradan have been demonstrated in plants, so that schradan could be considered endometatoxic. Metcalf (54) has stated that the thiol isomer of demeton is an effective systemic and is slowly detoxified to inactive components and could therefore be considered endolytic.

When using a systemic insecticide, the plant instead of being a passive spray target becomes an active participant in the subsequent processes which govern the efficiency of the application. The plant being a living system is in a continuous state of physiological and biochemical flux so that considerable variations occur. These variations exist not only between species but within a species at different times of the year, or possibly at different times of the day, and also under varying environmental conditions of light, temperature, humidity, nutrition, etc. It is probable that differences in some of these conditions are responsible for the variable results obtained with systemic insecticides which are reported in the literature.

The efficiency of a systemic insecticide is dependent on the active participation of the plant in three main processes, namely absorption, translocation, and detoxification. In this review absorption refers to penetration of the cell membranes by the insecticide, translocation to movements from

cell to cell, and detoxification to all methods encountered in the removal of toxic material from the plant. It is evident that plant physiology is the subject most intimately involved, but it is the one which has been neglected in systemic insecticidal studies.

ABSORPTION

It would appear that all external surfaces of plants are able to absorb some materials under certain conditions. It is essential, therefore, to consider the suitability of the various plant surfaces for the absorption of systemic insecticides.

By seeds.—The main practical advantage of absorption of insecticides by seeds is that protection against insect attack is afforded during the important seedling stage. It is obvious, however, that for such a method to be effective there must be a relationship between seed and plant size so that the seed is large enough to absorb sufficient insecticide to protect the resultant plant. Chao (13) found that plants of cotton, pea, various beans, and nasturtium grown from seeds soaked in solutions of schradan were successfully protected against attacks by aphids and red spider for up to 50 days, and generally the period of protection varied with the weight of the seed. Similar results were obtained on cotton and eggplants [Ashdown & Cordner (3)]. David & Gardiner (23) who worked with a variety of seeds, and a number of different chemicals, found that schradan and demeton could be successfully used on the larger seeds. They found that after 4 hr. soaking in the thiol isomer of demeton more toxic material was present in the seed coat than in the cotyledons, but that this was reversed after 24 hr. They also found that there was no preferential absorption of water or of the thiol isomer from solution, which would agree with the suggestion of Atkins (4) that the membranes of seeds of beans and sweet peas become semipermeable only after germination begins and that the forces concerned in the initial stages of water absorption are capillarity and imbibition before, and osmosis, after germination. Chao (13), on the other hand, found selective absorption of water from a solution of schradan by broad bean seeds, and the phosphorus content of the solution increased from 25 to 50 per cent.

By roots.—This is the normal channel of absorption of water and minerals into a plant from the surrounding media, taking place through the root hairs, which are specialized epidermal root cells. The composition of the cell walls and cytoplasm will be more fully discussed later as obviously this may well be the most important single factor affecting absorption. It is highly probable that all cells are basically similar but may vary in the proportion or arrangement of the various materials which so affects their permeability to different substances. From the work carried out on the application of systemic insecticides to the roots of plants, the most important factor is the contact between the roots and the insecticide. Experiments have demonstrated that the absorption of insecticides by roots from various media is greatest from solution, less from sand, and least from soil. David

(19, 20) found that more schradan and dimefox is absorbed from sand than from soil, and, it has been shown, that while demeton is absorbed freely from solution, the absorption from sand and humus containing soils is a slower process and is effected only up to a certain limited concentration, which differs for each type of soil [Teitz (66)]. It would seem, therefore, that differences in absorption from various media can be explained on a physical basis, either as a result of the varying contact between insecticide and roots, or of the greater affinity of the insecticide for some particles in the soil or solid media. The selective absorption or rejection of the insecticide by the roots in a standard medium is even more interesting. David (19, 20) found that from an aqueous solution of dimefox the roots of broad bean plants selectively absorbed the insecticide, but from a similar solution of schradan the insecticide was preferentially rejected, and it should be noted that the material with a lower lipoid solubility was more readily absorbed. Casida, Chapman & Allen (11) found that in pea plants there was a correlation between the absorption of schradan and the available inorganic phosphorus. Teitz (66) found that for the first day there was preferential absorption of demeton but after this time it returned to a standard value. He also found that the roots of *Phaseolus vulgaris* in nutrient solution, to which had been added 0.01 per cent demeton, absorbed far more water than the controls for the first 2 hr., but this increased permeability slowly diminished until after 8 to 9 hr. it was back to normal. He has suggested that intensified respiration is the probable explanation of the increased permeability. It is of interest in what manner this material could affect the physiological process of respiration and how this in turn could alter the permeability. Systemics of the urethan type, Dimeton and Pyrolan, were, however, shown by Gasser (34) to depress the rate of carbon assimilation in *Elodea canadensis* so that it practically ceased after one to two days. When the plants were returned to water after four days, assimilation returned to normal, and in this case no effect was observed on growth, division of cells, plasma movement, osmotic equivalent, respiration, or transpiration.

By leaves.—Whilst the leaves may not be considered normal absorptive areas of the plant, absorption can occur, and it has been found of considerable value, particularly in rectifying certain nutritional problems. When a systemic insecticide is applied to the foliage of plants a number of processes, such as evaporation, absorption, and breakdown, can go on simultaneously or sequentially. From the practical point of view, the relative importance of these processes will affect the ultimate behaviour of the material. The amount lost into the atmosphere by evaporation will depend largely on the vapour pressure of the insecticide and the physical conditions prevailing at the time of application; under conditions of high temperature and air turbulence evaporational losses may be considerable. Heath & Llewellyn (44) have quoted a loss of 50 per cent of applied schradan from brussel sprouts under field conditions, and the loss from plain surfaces was 10 times as great in highly agitated atmospheres as in still ones [Heath, Lane & Llewellyn

(43)]. Evaporational losses should obey the normal physical laws providing that the covering layer of insecticide is sufficiently thick. Bennett & Thomas (8) found that, when comparing the evaporation from glass plates and leaves, the loss from glass plates was about five times greater than from leaves, although, at the end of the experiment the amount of insecticide that could be removed from the leaf by washing with water was sufficient to cover it with a film of 50 molecules thick. They concluded that there was some form of mutual dissolution of the schradan and the lipophilic leaf materials which was sufficient to prevent evaporation but did not prevent the removal of material by aqueous leaching. The importance of volatility is demonstrated by dimefox which is volatile and of low lipoid solubility and is not a good systemic insecticide when applied to the leaves. Demeton is extremely volatile and toxic in the vapour phase, and a fumigant action has been demonstrated by a number of workers [Zattler (74); Lusis (50); Davis & Sessions (24); David & Gardiner (22); Thomas, Bennett & Lloyd-Jones (68)]. The fumigant action is of only short duration, probably because of breakdown into less volatile materials on the leaf surface, a process found to occur on filter paper by Cook (15). It is obvious, therefore, that evaporation can be an extremely important factor in the behaviour of systemic insecticides when applied to the aerial portion of the plant. If the vapour is toxic to insects, then a high initial kill may be obtained by fumigant action on certain crops under favourable conditions, but the loss of material by evaporation reduces the amount available for absorption and for subsequent true systemic action.

Before discussing the physical factors likely to affect absorption it is necessary to consider the most likely path of penetration into the leaf. The main modes of entry of materials into plant foliage are (a) cuticular [Weaver & DeRose (71); Fogg (30)], (b) by liquid penetration of the stomata by some of the petroleum oils, the rate being dependant on the surface tension and viscosity [Knight & Cleveland (48)], and (c) by vapour phase entry through the stomata. Stomatal entry of demeton has been suggested by Zattler (74) from observations on the increased period of residual toxicity in hop leaves when treated on the under surface as opposed to treatment of the nonstomate upper surface. Entry by cuticular penetration was suggested by Heath & Llewellyn (44) from the results of experiments with five different insecticides and allied compounds on *Brassica*, when they found that absorptive behaviour did not bear any close relationship with vapour pressure but was more or less inversely proportional to molecular size. Bennett & Thomas (8) agreed with this suggested mode of entry for schradan and it seems the most likely path for all systemic insecticides.

Before absorption can be said to have occurred, the systemic insecticide must be present in living cells and for this, penetration of the cuticle, the cell wall, and the plasma membrane are essential. Frey-Wyssling (33) has described the cuticular layer as composed of a mixture of the four cell wall constituents, cellulose and pectins which are hydrophilic and predominate

on the inside, cutin which is semihydrophobic, and cutin waxes which are lipophilic and predominate on the outside. The cell wall which is composed of cellulose fibres and pectic materials does not usually present much of a barrier to the penetration of substances, but the plasma membrane possesses the property of differential permeability. Collander (14) has stated that the plasma membrane or plasmalemma which is the outer boundary of the cytoplasm is a lipoid layer free of proteins, and Danielli (18) estimated that this layer was only two to four molecules thick because of the disappearance of semipermeability with a certain increase in surface size; however, this would not seem to be true in all cases [Curtis (16)]. Cytoplasm is composed of hyaline ectoplasm bordering the cell wall, its outer boundary formed by the plasmalemma rich in lipoids, whilst the endo or inner plasm consists of plasm gel at its periphery and the central part of plasm sol, the whole being intersected by strands of higher density [Scarth (62)]. The permeability of these layers of the cytoplasm varies with a number of factors. Marklund (52) observed that striking permeability changes occurred spontaneously during the normal course of development of the leaf cells of *Elodea* and *Taraxacum*. Permeability to glycerol increased 9 times and that to urea 46 times during the early development of the *Elodea* cells; in older leaves the permeability again decreased. The variation in absorption of schradan by bean and chrysanthemum leaves of different ages has been demonstrated by Bennett & Thomas (8) who found that the young leaves absorbed most and the middle aged leaves least. Similar observations have been made by a number of workers, but no quantitative data is available. Permeability also varies with the acidity of the plasma membrane lipoids. It is known that the addition of an organic acid to a neutral oil increases markedly the solubility of amides in the oil, thus when some cells are especially permeable to amides it is assumed that the plasma membrane lipoids of these cells are particularly acid in character.

The effects of temperature on permeability have been theoretically explained by Davson & Danielli (26) who have described the plasma membranes as a potential barrier through which only molecules having more than a critical energy of translation are able to pass. The value of this critical energy will vary directly with the resistance encountered by the molecule in penetrating the film. An increase in temperature will increase the molecule energy and therefore make possible the passage of a greater fraction of the total number of molecules in a case where the critical energy is large. The effect of temperature on permeability may not be immediately apparent as permeability of root cells to water is only gradually decreased by a sudden cooling from 20° to 0°C. [Döring (28)]. Levitt & Scarth (49) found that frost resistance and permeability varied together in the life of the plant not only in the normal seasonal rhythm and in relation to temperature changes, but also in response to other factors such as water supply, nutrition, and even disease. While there were conspicuous permeability changes to polar nonelectrolytes with small molecules, such as water or urea, there was no change

to apolar substances such as urethan. Heath & Llewellyn (44) have reported that incident radiation had a profound effect on the rate of uptake of schradan and bis-isopropylaminophosphorous fluoride, the higher the intensity the faster the uptake. As both visible and infrared radiation increased the rate of uptake about tenfold, they concluded that the cause might be thermal. This was not an effect on the whole plant but rather that the radiation incident on the leaf surface set up a temperature gradient near the surface which greatly increased its permeability. The absorption rate at 16 to 17°C. in a glasshouse in February was only about one-tenth of that of plants exposed to April sunlight in an unheated glasshouse at the same temperature. When *Brassica* were exposed to bright sunlight for several days they became highly absorbent and only lost their permeability slowly, and a week later were far more permeable than plants which were not exposed. This observation would appear to contradict the idea of a temperature gradient at the leaf surface and would suggest that incident radiation can have a lasting effect on permeability. Bennett & Thomas (8) found that light and heat could be important factors in absorption, which, in beans, could be increased by raising the temperature, even when the plants were screened from any direct radiation, but this was an effect on the rate and not on the ultimate capacity. They found that at higher temperatures daylight did not increase the rate of absorption whilst at the lower temperatures it did, and at both temperatures light seemed to increase absorptive capacity. It is probable that a large number of factors are involved in producing optimum conditions for plant processes and the relative importance of any individual factor at any time will be judged by its ability to make conditions approach the optimum.

These are important factors in absorption because of their effect on membrane permeability, but the physical nature of the surface to which the insecticide is applied is also important. Absorption by upper and lower leaf surfaces has been shown to vary [Zattler (74); Bennett & Thomas (8)]. The latter found that whilst some plants such as *Coleus* and broad bean showed similar absorption of schradan from upper and lower leaf surfaces, others like chrysanthemum and apple absorbed more via the lower leaf surface than the upper. In none of the plants tested was the upper leaf surface more absorptive. In these experiments the amount of insecticide applied per unit area was the same in each case, whereas Teitz (66) sprayed to run-off on hops and found that whilst absorption of demeton calculated from the amount in or on the leaf was only 10 per cent higher, the amount that actually penetrated was four times greater on the lower surfaces. This is explained by the presence of leaf hairs and the uneven surface formed by vein protrusions on the under surface of the leaf, not only increasing the surface area but providing suitable sites for spray adhesion. The spray usually runs off the upper epidermis and being more exposed, evaporational losses may be greater, so that the time the material is available for absorption is rather less than on the lower surface. However, when the above variables were

eliminated in short term absorption experiments with schradan on chrysanthemums, the lower surface was found to be about five times as absorptive as the upper [Bennett & Thomas (8)]. The lower leaf surface is generally more absorptive than the upper probably because cuticular absorption is not uniform over the whole leaf. The stomatal ridges, the basal cells of hairs, and the anticlines of the epidermal cells have been shown to be sites primarily concerned in cuticular transpiration and excretion of organic compounds and these or similar areas may be the ones involved in absorption. Teitz (66) found that the leaves of *Primula obconica* absorbed almost three times as much demeton as those of *Cyclamen persicum* which he explained as attributable to the total amount adhering to the hairy surface of the primula as opposed to the leatherlike cuticle of the cyclamen. In fact, his figures show that the ratio of absorbed to leachable, irrespective of amount originally adhering, is greater in the primula, indicating a difference in absorption also between species.

Few quantitative experiments have been carried out to determine the extent of absorption as a function of the applied material, evidence for the efficiency of absorption being deduced largely from the biological effectiveness of the application. Most of the work carried out with radioactive materials has assumed that any material recovered from the leaf after it has been subjected to washing in an aqueous solution had been absorbed [David (19), Teitz (66), Batt, Bennett & Thomas (5)]. It is possible that a high proportion of the insecticide assumed to be absorbed, may be in the cuticular layer and may not have penetrated the cells. Bennett & Thomas (8) and Thomas, Bennett & Lloyd-Jones (68) have quoted figures for the amount of schradan and thiol isomer of demeton removed from sprayed leaves by chloroform, demonstrating the presence of quite large quantities of these materials in the cuticle.

It is obvious, therefore, that the amount of any systemic insecticide that is absorbed following leaf application is dependent on a large number of factors which are closely interdependent. Such factors as time of year, leaf age, leaf surface, leaf type, conditions of temperature, and radiation have all been shown to play important roles in controlling the proportion of the applied insecticide that is absorbed. The initial effect of these factors is on the amount of insecticide retained per unit area of leaf, but probably their most important effect is that on cell permeability.

By bark.—The method of applying systemic insecticides to the bark of trees has been successfully employed in the case of demeton and schradan [Jeppson, Jesser & Complin (47)]. Bond (10) found that this method was more efficient than root absorption with dimefox on coffee, and the leaves of sour orange seedlings accumulated schradan at the same rate following bark application as following root application, even though less material was applied in the former case [Metcalf & March (56)].

The absorption of both lipoid soluble and nonlipoid soluble materials seems to be effected by this method, but Bond (10) did find that the ab-

sorption of dimefox was better when the material was applied to the exposed cambium of coffee trees, so it is possible that the outer epidermal or collenchyma cells are able to absorb the lipoid soluble materials more efficiently. The trunk implantation method as used by Hanna, Judenko & Heatherington (39) for the control of insects transmitting swollen shoot virus of cacao can be included here. In this method the insecticide is mechanically introduced into the trunk of the tree by a method similar to that used for introducing salts into trees to counteract mineral deficiencies.

TRANSLOCATION

Normally translocation implies the movement of materials in the phloem or xylem conducting tissue of the plant. As only the insecticides which are moved at some time or other in these conducting tissues are being considered, it is desirable to include all movement of insecticide following the initial absorption under the heading of translocation. The systemic insecticides depend for their efficiency on the extent, both in amount and direction, of their distribution through the plant.

The state of knowledge on the movement of both inorganic and organic materials within plants might be demonstrated by quoting from two fairly recent plant physiological reviews on the mechanisms involved.

Beyond the fact that ions are moved across the root by a mechanism dependent on accumulation, and liberated into the xylem, where they can move with the diffusion gradient or with the transpiration stream, we know little of movements of nutrients in the plant. Ions can move in the phloem: this path is probably important in the remobilisation of nutrients which occurs in the plant but we are ignorant of the mechanism.

[Robertson (61)]. On the transport of organic compounds, Arisz (2) states

It may be expected that transport in the sieve tubes is not fundamentally different from that in parenchyma in as much as the sieve tubes are more or less differentiated parenchyma cells. In the specialized older sieve tubes the method of transport, such as mass flow of the sap may prevail. . . . Little is known about transport in parenchyma cells especially of organic substances. The movement can take place in different parts of the cells, in the walls, in the protoplasm and in the vacuole.

This demonstrates the lack of knowledge of the movement of materials associated with plant growth and metabolism which makes the interpretation of the movement of systemic insecticides even more difficult. Once again, much of the published work dealing with translocation of systemic insecticides is from observations of biological effectiveness or failure. A limited amount of quantitative work has been done using radioactive isotopes, but the problem is further complicated by the breakdown of the insecticides, which is known to occur within the plant and will be discussed in the next section. Unfortunately the amount of work involved in determining the extent of the decomposition of insecticide in all plant samples taken from an experiment is so great, that in many cases, only a figure of total radioactivity in a particular section of the plant is available. One is, there-

fore, in some doubt as to the type of compound which is being translocated to any particular part of the plant.

It might be expected that the process of translocation will vary according to the insecticide and the site of absorption.

After seed absorption.—Following absorption by seeds it is to be expected that the cotyledons act as the reservoir of insecticide as well as plant foods, and parallel transportation to areas of new growth might be expected. Chao (13) using schradan showed that toxicity to aphids was first lost by the young leaves at the top of the plant demonstrating that the chemical was not evenly distributed but tended to remain in those parts to which it was originally translocated from the cotyledons. David & Gardiner (23) found that the distribution of the thiol isomer of demeton in the plant was fairly uniform after seed treatment and that translocation of insecticide occurred to the aerial part of the plant, some directly from the cotyledon and the rest from the roots after having passed into the soil and been reabsorbed.

After root absorption.—Translocation to the aerial parts of the plant from the roots occurs, often quite rapidly. It seems to be assumed by many that this is a passive movement in the transpiration stream. If this is the case then accumulation could be expected to occur in the areas of most active transpiration. Bennett (6) found the rate of translocation of dimefox in willow following absorption by the roots to be about 11 cm./hr. and suggested that translocation occurred in the xylem tissue, as restriction of transpiration of the leaf either prevented the insecticide reaching or being given off by the leaf. Teitz (66) found that demeton absorbed via the roots moved at a rate of 3.2 m./hr. in *Vicia faba* when the plants were kept at 21°C., 60 per cent relative humidity, and the intensity of illumination 10,000 lux. Under similar environmental conditions the rate of movement in *Salix viminalis* was 80 to 90 cm./hr. in still air and 120 cm./hr. in turbulent air. Wedding & Metcalf (73) found that the rate of movement of schradan in the stem of *Phaseolus vulgaris* varied from 17 to 58 cm./hr. with the majority at 20 cm./hr., and that the translocated material tended to accumulate more rapidly in the younger tissues of both stem and leaf. No attempt was made in their experiments to determine whether movement was in xylem or phloem, but they have suggested that the rate of movement was approximately that determined for the movement of organic materials of various types in the phloem [Curtis (17)]. Teitz (66) assessed the translocation of demeton in *Vicia faba* and *Salix viminalis* by analysis of various plant parts after different time intervals and concluded that the insecticide moved primarily in the xylem of the shoot axis and the leaves and accumulated in the peripheral zones of leaves as a result of the blind termination of the transpiration stream in the parenchyma cells. Limited lateral diffusion from xylem to phloem occurred, but the concentration in the phloem parenchyma increased only slowly.

Metcalf & March (56) in experiments on lemon seedlings with labelled schradan and phosphoric acid found that the distribution of both was sur-

prisingly uniform throughout the plant although schradan accumulated more in the median leaves while phosphoric acid was more concentrated in the basal ones. Autoradiographs of individual leaves showed a general distribution of phosphoric acid but a concentration of schradan in the most rapidly growing portions. This shows that the translocation, which would appear likely to occur in xylem tissue, can be selective and is not entirely dependent on the amount of transpiration occurring from a particular area. Metcalf *et al.* (57) found that the translocation of the thiono isomer of demeton in lemon seedlings was similar in amount and direction to that of schradan though it tended to accumulate rather more in terminal leaves.

After leaf absorption.—Generally the actual amount of insecticide translocated after leaf absorption is not great but the amount and direction seem to be more variable than after root absorption.

Metcalf & March (56) found that between 0.1 and 1.0 per cent of the total dosage of schradan applied to a single lemon leaf appeared in other leaves after 17 days. Thomas & Bennett (67) found about 1.0 per cent per day of the absorbed schradan or metabolites was translocated in beans, coleus, and chrysanthemums and up to 4 per cent per day in apples. The amount and direction varied according to the foliage zone treated, more movement occurring from older to younger leaves than in the reverse direction, although small quantities of undecomposed schradan were detected in leaves below those treated in apples and chrysanthemums. In contrast to the general observations, greater translocation of schradan to leaves below those treated has been demonstrated in *Vicia faba* [Zeid & Cutkomp (75)]. Thomas & Bennett (67) suggested, from results obtained by ringing apple rootstocks above and below treated leaves, that limited movement of schradan and its decomposition products occurred in an upward direction in xylem, but the majority of movement was in phloem and downward movement was exclusively in this tissue. This suggestion of movement of schradan in phloem tissue is supported by its presence in the nectar secretion of some plants [Glynne-Jones & Thomas (36)]. Thomas & Bennett (67) observed that light was an important factor in promoting translocation, for when plants were kept in darkness before and after spraying only very slight translocation occurred. Plant species varied in their behaviour, thus in *Phaseolus multiflorus* translocation occurred only in the presence of light whereas in *Vicia faba* and *Coleus* translocation occurred in the dark provided the plants had been in the light previously. The effect of light is probably not direct but depends on the fact that in some plants active photosynthesis, and in others the products of this process, are necessary for translocation of insecticide. Light and the photosynthate concentration of leaves has been shown to be important for the toxicity of 2,4-D in red kidney beans [Davis & Smith (25)]. Wedding (72) found a marked diurnal effect in both the direction and rate of translocation of demeton in lemon plants and has suggested that it may be an effect of light.

A number of workers have been unable to demonstrate the systemic

properties of demeton after leaf absorption, but others have shown that translocation, mainly to the leaf periphery, does occur but the veins and the cells surrounding them form a barrier to movement. This concentration in the leaf periphery suggests that movement in xylem is much easier than in phloem. In cotton translocation of demeton occurred only in the xylem, and although movement was traced in both directions, it was most rapid towards the apex [Ahmed *et al.* (1)]. Teitz (66) found that up to a certain percentage of the active ingredient was translocated, so that the more extensive the treatment of the leaves, the greater the amount translocated. Concentrations of demeton were found in epidermal cells and the cells of the connecting bundle parenchyma, and he suggests that when the concentration in these parenchyma cells rises beyond a certain limit, the insecticide is flushed passively into the phloem and from there, on a small scale into xylem; in *Pelargonium zonale* twice as much was transported in phloem as in xylem.

After bark absorption.—Wedding (72) found that after bark absorption of demeton by lemon plants, the initial translocation took place in the phloem, although diffusion into and transport in the xylem took place later. The rate of downward movement was 2.5 cm./hr. and of upward movement was 10 cm./hr. Little lateral movement of dimefox was shown to occur following its implantation into the trunks of cacao trees although upward movement in xylem occurred quite freely [Hanna, Judenko & Heatherington (39)].

DETOXIFICATION

As most of the systemic insecticides possess a fairly high degree of mammalian toxicity, it is important, if they are to be used on edible crops, that detoxification of the plants should occur, but it is equally important if they are to be efficient insecticides, that it should not take place too rapidly. Detoxification can occur in two ways, either by loss of the toxic agent from some plant surface or by decomposition of the toxic material by the plant into less toxic or nontoxic metabolites. Several processes have been reported to be involved in plant detoxification, and their importance seems to vary with the species, the insecticide, and the method of absorption.

David & Gardiner (23) demonstrated loss of the thiol isomer of demeton from treated seeds into the soil. Although some of this material was subsequently reabsorbed by the roots considerable losses occurred. By restricting the volume of soil smaller seeds were rendered insecticidal and larger seeds showed a greater residual toxicity.

Stein, Alper & Anderssen (65) reported that roots of groundnut (peanut) plants lost appreciable quantities of schradan following leaf application, but Thomas & Bennett (67) found little loss of schradan or its metabolites from the roots of *Vicia faba*. Teitz (66) found considerable quantities of demeton were exuded from the roots of *Vicia faba* plants following absorption by the

roots. These observations suggest that where the concentration of insecticide in the root is high such as occurs following root absorption, loss of insecticides or their metabolites may occur, but losses of translocated material from the roots will be generally of only small proportions.

Vapour loss of the volatile insecticides dimefox and bis(2-fluoroethoxy) methane has been shown to occur from leaves following root absorption, and it was suggested that they were transpired with the water vapour [Bennett (6)]. A fumigant effect following demeton applications to the leaves has been demonstrated by a number of workers, but the short dura- tion of the action suggests that it is of applied and not absorbed insecticide. Metcalf and co-workers (57) could find no evidence that translocated deme- ton or its metabolites were transpired from either lemon or bean plants fol- lowing stem applications although quite large quantities of the thiono and thiol isomers were present in the leaves. Teitz (66), on the other hand, found that insecticidal amounts of demeton were transpired from the leaves follow- ing root absorption and that it was independent of the number or the condition of the stomata. From this he concluded that the sites of exudation were the anticlines of the epidermal cells, the basal cells of hairs, and the stomatal grooves and that transpiration through the stomata was of second- ary importance. In his experiments loss to a certain level of concentration occurred quite rapidly, after which the rate of loss was reduced. It seems probable that following root absorption the volatile thiol isomer of demeton may be translocated to and given off from the leaves, but following leaf application these volatile materials are not translocated [Thomas, Bennett & Lloyd-Jones (68)]. The less volatile materials may be lost from the leaves under natural conditions by leaching as are some of the mineral elements. No evidence is available that absorbed schradan is given off from the leaf in the vapour phase, but loss by gutation has been suggested [Park & Heath (59)].

Whatever losses of toxicity occur by these means the most important and consistent, particularly in the case of demeton and schradan, is the metabolic breakdown of the material by the plant. Hartley & Heath (41) suggested that in the case of schradan enzymic oxidation followed by hy- drolysis occurs, and according to Casida *et al.* (12) the toxic phosphoramide oxide produced by oxidation may be responsible for phytotoxicity by its effect on the phosphatase enzyme system of the leaf. The presence of the phosphoramide oxide has been demonstrated in lettuce plants [DuBois, Doull & Coon (29)] and in beans [Hall, Stohlman & Schechter (38)], but it is so unstable that the amount present at any time is of trivial significance in producing the insecticidal effect, especially as the insect tissue can produce the metabolite much more rapidly than the plant. The fact that the same product can be produced chemically in plants, in mammals, and in insects shows that the breakdown is one of simple oxidation. Unfortunately little work has been done under controlled conditions to determine the factors

affecting the breakdown of schradan in plants. Heath, Lane & Llewellyn (43) have stated that the breakdown rate in all crops studied was the same but that variations occurred within a crop at different times of the year, breakdown being much more rapid in the summer than in the autumn. This would certainly agree with the long lasting insecticidal effect observed by Dicker (27) on the autumn treatment of strawberry runners with schradan. Bennett & Thomas (9) found widely varying breakdown rates in different plant species, it being quite rapid in beans and very much slower in chrysanthemums and coleus, while Metcalf & March (56) concluded that only a small fraction of the absorbed schradan in lemon seedlings was metabolised in the plant in 46 days. The metabolism of P^{32} labelled schradan after absorption by the roots of *Phaseolus vulgaris* has been described by Turrell, Weber & Storherr (69). From analysis of the trichloroacetic acid soluble and insoluble fractions they found that after five days of absorption of schradan, increasing amounts of P^{32} were present in the phospholipides, ribose nucleotides of small leaves, and in the ribose nucleotides and deoxyribonucleic acids of large leaves. Autoradiographs showed the highest concentration of P^{32} in the parenchyma cells of large trifoliate leaves while stems, petioles, veins, and small trifoliate leaves contained low concentrations. The metabolism and position in the plant of P^{32} from schradan bore no resemblance to that of P^{32} from disodium monohydrogen orthophosphate absorbed under the same conditions. It is probable that the oxidizing enzyme system in plants which is responsible for the initial breakdown of schradan varies in efficiency not only between species but within a species at different seasons. No evidence is available that any particular organ of the plant is concerned with breakdown although concentrations of the oxidizing enzymes may occur in specialized cells or areas of cells in various organs. The high concentration of undecomposed schradan found in the oily seeds of sprayed cotton plants by Metcalf and co-workers (55) could be explained by the absence of oxidizing enzymes or by the great affinity of schradan for oil which might prevent the oxidation of the insecticide.

The chemistry of the breakdown of demeton is more complicated. Hartley (40) and Heath, Lane & Park (42) have shown that the thiono isomer changes to the thiol isomer and suggest that the insecticidal action of demeton is attributable largely to the latter. They found the thiol isomer unstable after application to plants and by solvent partition procedure isolated three metabolites D_1, D_2, and D_3 from plants treated with the thiol isomer and attributed insecticidal activity mainly to D_1 and D_2. Fukuto and co-workers (31, 51, 57, 58) in an excellent series of papers have shown how they synthesized seven oxidation products of the thiono and thiol isomers and compared them with the metabolic products of demeton found in plants sprayed with demeton. From the results obtained from paper chromatography, cholinesterase activity, systemic activity, mammalian and insect toxicity they suggested the following pattern of breakdown.

$$
\begin{array}{ccc}
\text{C}_2\text{H}_5\text{O} \quad \text{S} & \text{C}_2\text{H}_5\text{O} \quad \text{S} & \text{C}_2\text{H}_5\text{O} \quad \text{S} \\
\text{P} & \rightarrow \quad \text{P} \quad \text{O} & \rightarrow \quad \text{P} \quad \text{O} \\
\text{C}_2\text{H}_5\text{O} \quad \text{OC}_2\text{H}_4\text{SC}_2\text{H}_5 & \text{C}_2\text{H}_5\text{O} \quad \text{OC}_2\text{H}_4\text{SC}_2\text{H}_5 & \text{C}_2\text{H}_5\text{O} \quad \text{OC}_2\text{H}_4\text{SC}_2\text{H}_5 \\
& & \text{O}
\end{array}
$$

I. Thionophosphate	II. Thionophosphate sulfoxide	III. Thionophosphate sulfone

$$
\begin{array}{ccc}
\text{C}_2\text{H}_5\text{O} \quad \text{O} & \text{C}_2\text{H}_5\text{O} \quad \text{O} & \text{C}_2\text{H}_5\text{O} \quad \text{O} \\
\text{P} & \rightarrow \quad \text{P} \quad \text{O} & \rightarrow \quad \text{P} \quad \text{O} \\
\text{C}_2\text{H}_5\text{O} \quad \text{OC}_2\text{H}_4\text{SC}_2\text{H}_5 & \text{C}_2\text{H}_5\text{O} \quad \text{OC}_2\text{H}_4\text{SC}_2\text{H}_5 & \text{C}_2\text{H}_5\text{O} \quad \text{OC}_2\text{H}_4\text{SC}_2\text{H}_5 \\
& & \text{O}
\end{array}
$$

IV. Phosphate	V. Phosphate sulfoxide	VI. Phosphate sulfone

$$
\begin{array}{ccc}
\text{C}_2\text{H}_5\text{O} \quad \text{O} & \text{C}_2\text{H}_5\text{O} \quad \text{O} & \text{C}_2\text{H}_5\text{O} \\
\text{P} & \rightarrow \quad \text{P} \quad \text{O} & \rightarrow \quad \text{P} \quad \text{O} \\
\text{C}_2\text{H}_5\text{O} \quad \text{SC}_2\text{H}_4\text{SC}_2\text{H}_5 & \text{C}_2\text{H}_5\text{O} \quad \text{SC}_2\text{H}_4\text{SC}_2\text{H}_5 & \text{C}_2\text{H}_5\text{O} \quad \text{SC}_2\text{H}_4\text{SC}_2\text{H}_5 \\
& & \text{O}
\end{array}
$$

VII. Thiolphosphate	VIII. Thiolphosphate sulfoxide	IX. Thiolphosphate sulfone

They concluded that thiolphosphate sulfoxide and the thiolphosphate sulfone are probably the principal toxic metabolites produced by plants. Both isomers and their toxic metabolites are subsequently hydrolysed to nontoxic diethyl phosphoric acids and alcohols, but the thiol isomer metabolites were found to persist in leaf and fruit tissues about twice as long as the thiono isomer metabolites. In general outline the biochemical mechanisms followed the same pattern in animals as in plants, the major differences were found in the rates of metabolism and degradation which were greater in the mammal than in the insect and greater in the insect than in the plant.

CONCLUSIONS

The insecticidal efficiency of the systemic insecticides is governed by the physiological and biochemical processes of the plant. Lack of detailed knowledge has resulted in the inclusion of a number of factors affecting these plant processes under a general term, "conditions of active growth." In this review the relative importance of some of the factors has been discussed, and cell permeability would appear to be of major importance because of its influence on the physiological and metabolic processes.

As the plant is so intimately involved the standardisation of the "physiological status" of the plant material for experimental work is as important as the standardisation of the insecticides employed. For detailed comparative and quantitative studies plants should be raised under standardised

nutritional and environmental conditions and kept under carefully controlled conditions during the experimental period. By this means more information on the behaviour of systemic insecticides attributable to the various physiological and biochemical processes of the plant will be obtained. Although it is impossible to control all environmental conditions in the field, a better knowledge of the fundamental systems involved in the behaviour of systemic insecticides in plants would not only help in reducing the variability of results in field and laboratory work, but might also be useful to the chemist in the preparation of new insecticides.

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AERIAL APPLICATION OF INSECTICIDES[1]

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INTRODUCTION

While aircraft have been used for well over 30 years for the dispersal of chemicals to croplands little engineering or design data have been available until recently. A distinct handicap to previous engineering development has been the lack of facilities for the quantitative evaluation of chemical distribution and the determination of the effects of the complex variables affecting such distribution. In recent years such facilities have been provided by various state and federal research agencies, and attempts are being made to correlate the factors affecting aerial distribution. Since aerial application as a science is in its infancy, this paper must of necessity be a synopsis of some of the facets of its present status, from an aero-mechanical standpoint, rather than just a critical analysis of current literature. Other reports dealing with aerial application of insecticides written in a comprehensive manner and from different viewpoints, are given in two references of European origin (64, 65).

AGRICULTURAL AIRCRAFT

Adapted Types

Practically all aircraft now used in aerial application in this country were originally designed for other purposes, ranging from training to heavy bombing. Obviously their general arrangement, performance, and flying characteristics often differ widely from what is technically desirable for agricultural work. Even the new commercial aircraft presently available for aerial application work are largely modifications of previously existing designs; but new models designed throughout especially for the purpose are appearing in prototype form and should be available commercially complete with distribution equipment in the near future.

Large bomber and transport aircraft are used occasionally for insect control in large forest areas and over extensive rangelands, but the agricultural work in the United States is done mainly with two types of airplanes: light high-wing monoplanes and somewhat heavier biplanes, both used originally as trainers. Most of the biplanes are Boeing-Stearman models originally fitted with 220 horsepower engines; large numbers of them have been fitted with more powerful engines. The Stearman airplanes carry dust or spray loads ranging from 600 to 800 pounds with the smaller engines and up to about 1500 to 1700 pounds under favorable conditions with a 450 horsepower engine.

[1] The survey of the literature pertaining to this review was completed in July, 1956.

Most of the smaller high-wing monoplanes are modified Piper J-3 airplanes with larger engines (85 to 125 horsepower) in place of the original 65 horsepower one. The present 150 horsepower Piper PA-18A, which is the lineal descendant of the J-3, is being used in increasing quantities. The PA-18A is one of the few new airplanes now available commercially ready as delivered for aerial application. The low-powered high-wing monoplanes usually carry spray or dust loads of about 400 pounds, and the more powerful ones carry up to about 800 pounds.

The Callair A-5 and the Rawdon are examples of agricultural modifications of former passenger light low-wing monoplanes that have been adapted to aerial application. The Callair is now in small production. It originally seated two people side by side, and the hopper has been put in the place of the passenger on the right hand side.

Helicopters.—At present about 0.5 per cent of the aircraft used in aerial application in this country are helicopters. These are of post-war design, manufactured by three companies, Bell, Hiller, and Sikorsky. Although they were designed primarily for other purposes, in two cases the company furnishes dust and spray distributing equipment with the helicopters. Their initial and operating costs are high, and under present conditions their use appears to be justified economically largely in cases where they can do a good job which the airplane cannot accomplish satisfactorily.

Aircraft Designed Especially for Aerial Application

The Ag-1 experimental agricultural airplane.—The first airplane designed from the ground up for the purpose of distributing agricultural materials was the experimental Ag-1, which was developed in 1949 to 1950 at the Texas A. and M. Aircraft Research Center (1, 2). It was a low-wing monoplane of simple all-metal construction, carrying a spray or dust load of 1200 pounds and powered with a 225 horsepower flat six-cylinder Continental engine. The airplane was fitted with powerful full-span, high-lift flaps and special slot-lip ailerons which together made possible flight at exceptionally low speed with rapid response to aileron control. It was fitted with both a hopper in the fuselage for dust (27 cu. ft.) and tanks in the thick wing for spray fluid (150 gal. total), for change from dust to spray without any loss of time. The pilot's field of view was exceptional and considered adequate in every respect. At the same time the pilot was located to the rear of all loads and heavy masses, in a cockpit designed to protect him, and with a long and substantial structure ahead of him designed to fail progressively and reduce the shock in a nose-down crash.

Investigations over the past 10 years show that human beings can ordinarily withstand low-speed crashes without injury if adequate support and protection are provided (3, 4). Nearly all of the fatal accidents that have occurred in crop-control flying in this country during the past three years have been associated with either stalls or collisions with obstructions, usually electric wires or trees (5). It has been demonstrated that a man can with-

stand momentary accelerations of 40 g (40 times the man's weight, or approximately 8,000 lbs.) when properly supported (6, 7). And since the indications from safety-belt failures are that accelerations of 15 to 25 g are likely to be obtained in crashes of the kind under consideration, a harness capable of withstanding a 40 g acceleration appears well warranted. Adequate support for the harness, is, of course, necessary. Such a seat belt and shoulder harness arrangement was incorporated in the Ag-1 airplane (8).

Developments from Ag-1.—There have been two direct developments from the Ag-1. One of these, designated the Ag-2, is being prepared for manufacture by the Transland Company, Torrance, California. It uses substantially the same structure as the Ag-1 but incorporates a 450 horsepower radial engine and is to carry a load of 2000 pounds as compared with 1200 for the Ag-1. The other, which is referred to as the Ag-3, has, like the Ag-1, been designed and constructed by the Texas A. and M. Aircraft Research Center. It is an experimental airplane designed to carry a spray or dust load of 800 pounds with a 135 horsepower engine. It is smaller in size and cost and is fabric covered. In these two new designs we have airplanes carrying both larger and smaller loads than the Ag-1, with the same general over-all configurations and an attempt at the same pilot-safety characteristics with regard to field of view and structural arrangement for protection in crashes.

Other new agricultural airplanes.—Other entirely new agricultural airplane designs are being constructed in various parts of the country, and some of these will no doubt become available commercially. These include the Snow low-wing monoplane, which has good load carrying ability and low speed turning performance, and the Air Tractor biplane (9).

Another new special agricultural airplane is the Fletcher "Utility," manufactured in California (10). This airplane is an entirely new design of about the size and load carrying ability of the Ag-1. It is of all-metal construction and has a tricycle landing gear.

Two completely new designs have just been constructed in prototype form in England, both aimed at New Zealand usage. They are the Auster B.8 Agricola low-wing monoplane (11) and the Edgar Percival P.9 high-wing monoplane (12). Both show evidence of careful and thorough design approaches.

In summary the trend in new airplanes designed especially for aerial application has been as follows: (a) To decrease the likelihood of accidents through (i) ample field of view for the pilot and (ii) satisfactory lateral control and stability at the lowest speeds; and (b) to protect the pilot as far as possible in a crash.

GENERAL

AIRFLOW ABOUT AN AIRPLANE

The paths followed by the dust particles or the droplets of spray which are dispersed from an aircraft depend to a large extent upon the flow of the air about the aircraft. The smallest particles fall into the category of aerosols

and follow the airflow almost completely. The largest particles are heavy enough to fall through the air at fairly high velocity, but even their paths are influenced substantially by the motion imparted to the air by the aircraft.

In order to obtain lift as it moves forward through the air, an airplane must impart a downward motion to the air. The reaction obtained from accelerating the air downward is the lift. The downward motion of the air through which the wing has passed is called the downwash. The downwash is advantageous in that it helps to carry the particles down toward the ground and to lessen the time that they can be drifted out of position by wind.

The lift is transmitted to the wing through an increase in air pressure below the wing and a reduction in the air pressure above it. When viewed from the rear, the high-pressure air under the wing will tend to flow out and around the tip of the wing to the low-pressure region or partial vacuum above the wing. After the wing has passed, the air back of the tip continues to rotate for a considerable time in what is referred to as the trailing wing-tip vortex (13).

This action has been well shown in balloon studies by Akesson & Yates (14). The balloons were the same weight as the air they displaced and followed the motion of the air surrounding them after being released from an airplane with the wing about 10 ft. above the ground.

The motion of the air following the passage of an airplane can be determined by mathematical computation, even taking into account the effect of the ground when the airplane flies close to it (15, 16). These airflow computations for one airplane (Ag-1), as well as the theoretical trajectories of spray droplets dispersed from it, have been made by Reed (17).

The amount of air acted upon by the airplane is theoretically equivalent to that within the cylinder extending along the flight path and having the span of the airplane as its diameter. The smaller the span the smaller is the quantity of air moved downward, and the greater must be the downwash velocity.

The slower an airplane flies, and the more heavily loaded it is, the more rapid must be the downward displacement of the air in order to maintain sufficient lift to support the weight of the airplane. A slow flying or heavily loaded airplane, therefore, is accompanied by stronger downwash and stronger wing-tip vortices.

Distribution Measurement

A number of different methods are used for measuring the distribution patterns deposited from aircraft. Possibly the most direct method is that developed and used at the A. and M. College of Texas (18). It uses a number of delicate platform scales and weighs the material where it falls. The colorimetric method of quantity evaluation is used by the United States Department of Agriculture stations at Forest Grove, Oregon and Beltsville, Maryland (19, 20). Approximate methods for field studies have been worked out for making quick estimates of spray quantity by comparison with sam-

pler sheets representing known application rates (21). The above methods give the amount of material applied to a given area, but they give no information regarding the individual droplet sizes or spacing. To obtain this information the droplets are collected on various sampling surfaces and measured individually (22, 23).

The ultimate measure of the effectiveness of aerial application is the biological result, and in some cases it is possible to survey this with reasonable accuracy, i.e., the population counts of insects before and after treatment (19).

PENETRATION AND COVERAGE OF PLANT SURFACES

When the material is dispersed over trees or crops with dense foliage, it is often necessary to obtain a good deposit on the lower leaves. The downwash from the aircraft aids in obtaining good penetration; it disturbs the leaves, moving them around and turning them over, so that the material reaches the lower levels and will be deposited on the bottoms as well as the tops of the leaves. The downwash and the resulting disturbance of the leaves is greatest when the airplane flies as low and slowly as feasible.

In forest spraying, where the flying is usually done well above the tree tops, the best penetration appears to be obtained with relatively fine droplets (24). In like manner, the best penetration of dense cotton appears to be obtained with dust which has many very fine particles.

Whether or not a spray particle will impinge on a plant surface is dependent upon the mass of the particle and its velocity (momentum) as well as the relative size and shape of the surface. Brooks (25) has presented information that shows the relationship between the percentage of dynamic catch, droplet size, airstream velocity, and a $\frac{1}{8}$ in. cylinder. These data show that to achieve a dynamic catch of 50 per cent, droplets of 20 μ in diameter must be moving in an airstream with a velocity of 5 mph, while droplets of a 10 μ diameter require a velocity of greater than 20 mph. In addition, with an airstream velocity of 3 mph the dynamic catch of droplets of 10 μ and 20 μ diameters would be only about 7 per cent and 40 per cent, respectively. So the dynamic catch, or collection efficiency, varies as the droplet diameter or velocity or both and inversely with the diameter of the obstructing cylinder.

While it is desirable to have all the spray particles impinge on the plant foliage, it can readily be seen that if undercoverage of plant surfaces and penetration of deep foliage is to be obtained then a 100 per cent dynamic catch by the upper leaves or foliage is not desirable. Instead, it is necessary for a portion of the spray fluid to be in droplets small enough so that they filter down through and deposit underneath the canopy formed by the upper foliage.

DISTRIBUTION OF SOLIDS

EQUIPMENT DESIGN

General.—In most equipment now in use in the aerial distribution of solids, the material falls through a controlled opening or gate at the bottom

of a hopper into an enclosed air stream. The latter is sometimes in the form of a venturi tube which increases the air velocity and decreases the air pressure at the position of the gate where the material enters the air stream.

Though little engineering development has been done with solid distributors some general facts are known and can be considered. First, many tend to produce irregular flow. Often it takes several hundred feet of forward travel of the airplane before the full flow of material is established; in other cases the flow is pulsating with visible slugs of material coming from the distributor. The first difficulty can probably be attributed to the throat-agitator configuration, because in one instance it was overcome by the modification of the throat adjacent to the agitator (23). The second problem of pulsating flow with present distributors is a more general one and has been found to occur even with material as heavy as rice seed (26). It is conceivable that the difficulty is inherent in most present equipment; the mass flow rate of a material into a distributor is partially dependent upon the existing static pressure in that distributor, and yet the static pressure is also partially dependent upon the mass flow rate. In any event the use of an accurate metering device would eliminate much of the flow rate problem.

This fact has been recognized for some time, and as a result some of the commercial operators as well as various agricultural aviation research groups have worked on the problem. This includes the work of Henry (27) with variable speed metering devices, that of Sanders (28) with a dust fluidizer, and others.

Even with a controlled flow of material into the distributor other problems exist in distributor design because the drag of the individual particles emitted into the distributor slows down the airstream through it. This was well shown by a series of tests made by Henry (29) where 10 different distributor sections were tested in a wind tunnel. The tests, while being made with wheat seed, showed that the reduction in air stream velocity was approximately proportional to the amount of material metered into the distributor section. This correlates the experience of many operators who have found that overall swath widths tend to decrease with increased application rates of seeds and fertilizers. It was also shown quite definitely by Sanders (30) in series of fertilizer distribution measurements. Occasionally, with some distributors if the flow of dust or other solids is too great, complete "plugging" of the distributor occurs with subsequent spillage of material out of the front.

Dust.—The distance that any material can be dispersed laterally from a distributor is dependent upon the mass, drag, and velocity of the individual particles. Most dust is composed of very fine particles which have relatively high drag and little mass. As a result, even though the particles can be accelerated to high velocities by the airflow through the distributor, they lose their momentum almost instantly upon leaving it and are almost wholly subject to the external air movements. For this reason, the primary function of the present centrally-located dust distributor is to get the material out of the hopper and into the airflow about the airplane, and the airflow does the

lateral spreading. Quantitative evaluation of the distribution characteristics of some of the present dust dispersal equipment indicates that a substantial portion of the output comprising the heavier particles is deposited in a narrow strip below the center of the airplane. Visual observation does not always agree with this because many of the finer particles of dust are carried outward by the sidewash of the airplane and become involved in the wing-tip vortices, and it appears that a very wide swath is being obtained.

The limitations of present dust distributors can probably be overcome by one method or a combination of several methods. First, the use of larger, more uniformly graded particles would probably facilitate more uniform distribution and permit operation in higher winds. Second, wider swath distribution can probably be obtained by releasing the dust at points some distance from the center of the airplane, so that the airflow associated with the airplane's flight would be a greater aid to lateral dispersal.

Granulated materials.—In the strict sense granulated material encompasses a wide range of individual particle sizes from dust on up through sand-like particles to shot-size pellets.

The particle size of the sand-like granulated material may be a disadvantage in the effort to obtain maximum distribution width. This type of particle is enough larger and heavier than a dust particle so that the sidewise airflow about the airplane does not help substantially with lateral dispersion, and yet, it has insufficient mass to retain for long any momentum that might be imparted to it from a properly designed distributor.

The pelleted (shot-size) material offers some advantage in achieving maximum width of lateral dispersion. This class of material can be accelerated by the drag forces of the airflow through a distributor to velocities that, because of the large mass of the particle, gives a considerable kinetic energy. Then by directing the path of the particle outward, it travels some distance before the drag dissipates the kinetic energy and the particle falls to the ground.

Factors Affecting Distribution

In the distribution of solids the primary conditions or factors that affect distribution are (*a*) aircraft height, (*b*) aircraft speed, and (*c*) atmospheric conditions of wind and turbulence. The effect of these variables is interrelated, but for clarity they must be considered separately.

The effect of height.—The most general effect of an increase in height is an increase in pattern width, or lateral dispersion. Neglecting any wind effect, it has been found, however, that this increase is rather small. Although little quantitative measurement work has been done with dusts, it can be seen from distribution investigations with spray (31) that ordinary variations in height have relatively small effect on the pattern width.

The effect of airspeed.—It seems likely that the effect of airspeed on the distribution of solids depends primarily on the particle size of the material being distributed. With dust, since its lateral dispersal is primarily dependent upon the airflow about the airplane, the swath width should tend to increase

with a decrease in airspeed of a particular airplane because the sidewise airflow is increased. With large particle sizes the airflow about the airplane is not so important; of more importance is the velocity of the air through the distributor that accelerates the particles for lateral projection. In this case, increased airspeed should result in increased swath width.

The effect of wind.—In discussing the effect of wind it is necessary to consider that the wind has two components relative to the airplane. One component is parallel to the line of flight and the other component, crosswind, is at a right angle to the line of flight. In general, the wind parallel to the airplane's flight path has little direct effect on the distribution pattern. The turbulence associated with high velocity winds naturally affects the distribution to some extent, and any component of the wind parallel to the line of flight also results in a variation in ground speed on alternate flight directions and consequently affects the application rate.

Certainly, however, the cross component of the wind has a decided effect on the distribution of solids, particularly dust. Because of the drift problem with dusts they must be dispersed under relatively low wind (3 to 4 mph) and stable atmospheric conditions. This confines their dispersal to early morning and occasionally late evening when there is little turbulence and convection. A study of airplane dusting has been reported by Kruse, Hess & Metcalf (32). They reported that, under the conditions of early morning and a flying height of 20 to 30 ft., of the total dust dispersed "about 20 per cent falls in the central 100 ft. of the dusting swath" and altogether "about 28 per cent falls in a swath 200 ft. wide. . . . Thus over 70 per cent of the dust drifts away from the treatment area and for practical purposes may be considered lost." This may be an extreme case, but it typifies the drift problem inherent with small particle sizes.

Solid Particle Distribution Problems

Drift.—While drift is no problem with solids of large individual particle size, it does remain a problem with dusts. In addition, many of the newer ungraded granulated formulations contain substantial quantities of particles in the size ranges subject to drift. Even with supposedly nonhazardous dusts, drift constitutes a problem because of the high loss of material from the treated area.

It seems likely that drift can be reduced in two ways. First, a better understanding of the atmospheric conditions of inversion, convection, and turbulence would introduce limitations that would govern dust applications (33). Second, the use of solid formulations or materials with larger particle sizes and faster settling rates would alleviate the drift problem.

Brooks (25) states "In view of the three-fold disadvantage of very fine dusts—poor catch, low lethal power, and long distance drifting—it seems advisable to investigate the basis of the common axiom: 'The finer the particle the better the control.' "

Deposition.—This again is a problem that is confined almost solely to dust

(and small spray particles) and is directly related to drift. The deposition rate of small particles on plant foliage is apparently affected by a number of factors including leaf surface and temperature, humidity, the size and electrostatic charge of the particle, the particle velocity, and the airflow velocity and direction relative to the foliage surface. Considerable effort has been expended by various researchers studying these factors and evaluation techniques. (25, 32, 34 to 41).

Metering.—Rate of flow control remains the most pronounced problem in the application of all solids. Not only is the average flow rate (and application rate) dependent upon the pilot's experience and judgment, but it also depends on the flow characteristics of the material being distributed with the particular piece of equipment being used. This, coupled with the variations in longitudinal flow, makes the use of satisfactory metering almost mandatory.

DISTRIBUTION OF SPRAYS

DESCRIPTION OF SPRAY EQUIPMENT

General types.—Aircraft spray systems are generally classified as being either pressure or gravity feed types. The majority of the presently used sprayers are of the pressure type, incorporating wing-mounted booms and nozzles. The gravity feed type of system includes some boom arrangements used for herbicidal work, the rotating brush or disk dispersal devices, and multiple Venturi devices.

Tanks.—Tanks vary in size from 35 to 40 gallons in some light aircraft to over 200 gallons for airplanes equipped with a 450 horsepower engine. Of course, larger aircraft, such as the DC-3 and others used for forest insect control, use larger tanks, but there are relatively few of these airplanes in use.

The materials used in the construction of spray tanks varies considerably. Aluminum alloy is probably most widely used, but other materials include galvanized iron, stainless steel, fiber glass, aluminum lined with fiber glass, and aluminum with a synthetic liner. Recently there has been increased emphasis on the use of stainless steel and fiber glass because of their excellent corrosion resistance (43). The units employing synthetic liners have the advantage of interchangeability and the avoidance of contamination when changing spraying mixtures.

Spray pumps.—Three types of pumps have been most generally used with aircraft sprayers. They are the centrifugal, gear, and lobe types. The centrifugal pump is the most widely used because of its economy and availability, and because experience has proven that it fits the demand quite well. At normal operating speeds it supplies a large volume of fluid at spray pressures generally used with aircraft equipment. Also, it is not as susceptible as some other types to the abrasive effects of grit, dirt, and liquid suspensions (wettable powders).

The gear pump, because of its poor performance with small amounts of

wear and its pumping characteristics of high pressure with low volume, is used only to a limited extent.

The lobe-type pump has some of the characteristics of both the centrifugal and gear types. It has no mating surfaces as does the gear pump, hence it is usable with wettable powders; also, it can handle reasonably large volumes of fluid at most desired pressures. It does have the important disadvantages of probable greater weight and expense as compared to the other two pump types.

Spray pump drive methods.—The wind-driven installation is the simplest and most widely used type of spray pump drive method. The wind-driven pump has the disadvantage of being a relatively inefficient drive, but this is usually outweighed by its simplicity. Variations in airspeed result in changes in pump speed and spray pressure, unless a pressure regulating device is incoporated in the system.

With engine-driven spray pump installations the pump is occasionally attached directly to the accessory section of the engine, but more generally it is driven by means of V-belts or flexible shafts. A clutch or some other type of pump-disengage control is highly desirable.

Probably the most flexible and versatile spray pump drive method is that which uses a hydraulic linkage between the airplane engine and the pump (42). The spray pump may be located in any attitude or position with disregard to the physical limitations imposed by the mechanical or wind-driven methods. Spray pump speed can be controlled by the needle valve or the relative displacements of the pump and motor or both. In addition, other hydraulically-driven devices such as a dust agitator on the dust-hopper door mechanism can be linked into the system.

Lines and booms.—Because of corrosion (43) there appears to be a trend toward the use of plastic lines and booms; at present the most widely used materials are aluminum and steel tubing and occasionally, galvanized pipe and conduit.

Boom sizes, shapes, and locations vary considerably. Most booms are full span, extending from wing tip to wing tip and externally mounted below the under-surface of the lower wing on the biplanes and attached to a spar or a lift strut on the high-wing monoplanes.

Some operators, for drag considerations, use streamlined aircraft tubing for booms, and others mount the boom inside the wing with only the nozzles exposed below. All too often, however, booms, brackets, pumps, valves, and controls are located so as to result in excessive and unnecessary drag that must be overcome by additional horsepower.

A boom location that is apparently finding increasing favor among operators with airplanes with a low wing is that which has the boom supported about 4 to 6 in. aft of the wing trailing edge. Here, the boom is located in the wake of the wing and the drag should be less than with an underwing location. In addition, the nozzles can be seen from the cockpit and the boom is less likely to be caught on obstructions.

With much present equipment too little attention has been paid to the desirability of maintaing low line losses by using lines adequate in size, large radii turns, and a minimum of restrictions.

Nozzles.—The term "nozzle," as generally used, includes a wide variety of devices for controlling and dispersing the spray liquid in droplet form (44, 45). Some are manually operated by mechanical linkage, while others utilize spray fluid pressures for opening and spring loading for closing.

The manually operated nozzles have many configurations and for the most part have been fabricated in the operators' shops. Some are simple plug valves which incorporate on the downstream side either a commercially manufactured jet, or in some cases, as simple a device as a piece of flattened and bent copper tubing.

One low pressure dispersal device is the rotating brush, disk, or drum. These assemblies, and there are several types, utilize a wind-driven propeller on the forward end which turns a wire brush, series of brushes, or a number of disks attached to the aft end of a through shaft. The spray fluid flows by gravity from the tank to the dispersing unit. It is then fed onto the rotating brush or disk and thrown into the slipstream by centrifugal force. The rotating brush assembly achieves some control over volume and droplet size by varying the spacing between brush segments, the size of the individual wires, brush diameter, and rotational speed. The rotating disk unit has been used by the United States Department of Agriculture to distribute insecticides, some having the consistency of a viscous mud (46). It is felt that this type of unit can be used for any type of fluid that will flow by gravity.

An English development of the rotating disk principle is the rotating drum or cylinder. The outer surface of the cylinder consists of a fine screen. The cylinder is rotated by a V-belt drive from an adjustable windmill. The liquid is fed into the hollow cylinder, which is 4 in. in diameter and 14 in. long and thrown off through the openings of the surface screen as small droplets. Apparently, satisfactory results have been obtained by using only two of these units, one at each wing tip, when very small droplets are dispersed (47).

The pressure operated nozzles all utilize a spring loaded check valve which may be a ball, diaphragm, or poppet type. Normally, this check valve is an integral part of a nozzle assembly which includes check valve, screen, and appropriate orifice or jet. The diaphragm check valve is the most widely used and generally accepted pressure operated type nozzle assembly. The single nozzle most used is probably the type that incorporates the check valve, screen, and orifice all in the same body. The orifice or jet is quickly removable and interchangeable, resulting in the possibility of securing a wide range of volumes and spray patterns. The spray patterns may be fan shaped, a hollow cone, or a solid cone of several different included angles. This gives the operator quite a choice of possibilities to satisfy his particular needs.

There are many other nozzles in use at the present time, some of which are the result of the individual operator's ideas and wants, while others are

commercially available items. They differ in detail, principle, and construction, but so far all of them produce a range of droplet sizes greater than is desirable.

Miscellaneous units.—A flow meter or flow regulator would be an extremely desirable adjunct to most spray systems. To date few, if any, aircraft incorporate such a device, although industrial models are currently available. Future equipment development will probably result in the adaptation and incorporation of such units.

The use of suspensions and emulsions necessitates a continuous agitation of the spray mixture while it is in the aircraft. The principle means of accomplishing this to date has been through hydraulic agitation. Practically all aircraft sprayers are built to have sufficient pump capacity to be able to return a certain percentage of the pump output to the tank for this purpose. The amount of material required for adequate agitation is not known. It can be seen that the tank configuration, return inlet location, and the material being sprayed would all influence the requirements for proper agitation. Past experience and the increased use of suspensions and unstable emulsions make it appear that more definite and complete agitation will be required in the future. The most positive agitation can perhaps be accomplished with mechanical means by using rotating paddles or stirring devices.

Formation of Droplets from Aircraft

Aircraft spray equipment in general utilizes various types of industrial nozzles; mainly, these are the hydraulic or pressure nozzles. In consequence, droplet formation and size distribution is dependent upon all the factors that influence these nozzles in the static condition as well as the effect of dispersing a liquid in a high velocity airstream.

While a study of droplet formation from a static source is not directly applicable to aircraft spraying it does provide basic background information and can serve as a foundation for the study of droplet formation and dispersal under the conditions encountered in aircraft application.

Laboratory atomizers, producing drops within a controlled or limited size range include the micro-injection device described by Buck (48), the capillary tube with parallel airflow by Ennis & James (49), the vibrating glass capillary described by Dimmock (50) and Vonnegut & Neubauer (51), and the vibrating reed of Davis (52) and Rayner & Hurtig (53). Another laboratory device, with industrial and aircraft application as well is the previously mentioned spinning disk. Because of its wide application much laboratory work has been done with this type of atomizer. This includes the work of Walton & Prewett (54), Hinze & Milborn (55), Fraser & Eisenklam (56), Yates (57), Adler & Marshall (58) and others.

The effect of airspeed on droplet size.—It is apparent that the relative velocity of the fluid flowing from a nozzle, as compared with the airstream velocity flowing around the nozzle, depends upon the fluid pressure and the direction of the fluid flow. It has been shown that nozzles directed into an

airstream will result in a finer spray than the same nozzles directed with the airstream at the same pressure; the higher relative velocity reduces droplet size. In this vein, if the emission velocity of the fluid from a nozzle directed with the airstream is increased to the airstream velocity then the largest droplets would occur. This is true, however, only with a straight-jet type of nozzle because increase in pressure with most other nozzles used tends to decrease droplet size. Continuing in the same line it can also be seen that an increase in airstream velocity or, in the case of the aircraft sprayer, an increase in airspeed will produce smaller droplets. This has been substantiated by considerable testing (59, 60), including some which has been done at the Aircraft Research Center, A. and M. College of Texas (23, 61).

It is significant that the data from the latter tests indicated that for water the increase in airspeed resulted in a linear decrease in droplet size which was independent of orifice diameter. The m.m.d.[2] changed from 370 μ at 50 m.p.h. to 320 μ at 80 m.p.h. Such was not the case with oil, however; in this instance, while an increase in speed decreased droplet size, the larger orifice diameters produced larger droplets at the lower speeds, and it was not until an airspeed of 80 m.p.h. was approached that the droplet sizes for the different orifices were substantially the same (approximately 210 μ).

The effect of orifice diameter on droplet size.—While the effect of orifice size on droplet diameter was partially shown in the preceding data, it would be well to consider it somewhat further. Most aircraft spraying is done at speeds of 80 m.p.h. or greater so that the effect of orifice diameter is of greatest interest at these speeds. With a nozzle producing a hollow-cone type of spray, orifice diameters from $\frac{1}{16}$ in. to $\frac{3}{16}$ in. had no appreciable effect on median droplet size. This was true with water as well as with oil, even though the water m.m.d. was about one and one-half times greater than that of oil (320 μ and 210 μ, respectively).

The fact that, at an airspeed of 80 m.p.h. and a spray pressure of 35 p.s.i., variation in orifice diameter in the nozzle producing the hollow cone type of spray does not materially affect droplet size cannot be over-emphasized. As a result of it, a single nozzle arrangement giving satisfactory and biologically effective distribution will suffice for a wide range of application rates.

The effect of spray pressure.—To determine the effect of spray pressure on m.m.d. the airspeed and orifice diameter were held constant for a series of tests while the spray pressure was varied from 10 to 60 p.s.i., using oil and water (61). While water gave a considerably larger droplet than oil under the same conditions, both showed an approximately similar linear rate of decrease in

[2] Since all the presently used nozzles produce a relatively wide range of droplet sizes it is necessary to indicate the relative fineness or coarseness of the spray by some index. In general, the most commonly used index for agricultural spraying is the mass median diameter (m.m.d.) denoted in microns (μ). By definition this index is the figure which divides equally the total volume of spray; one-half the volume is in droplets smaller than the m.m.d., and the other half is in droplets larger than the m.m.d.

m.m.d. with an increase in pressure. This variation with pressure was relatively small (12 μ/10 p.s.i. for water, 10 μ/10 p.s.i. for oil) and indicated that in actual practice a wide range of pressures could be used to get a desired flow rate without radically changing the median droplet size or subsequent distribution pattern.

Summary of factors affecting droplet size.—Within the range of normal spraying practice the characteristics of the spray fluid, i.e., surface tension, density, etc., are probably the most important factors affecting the droplet spectrum. While the hollow-cone type nozzle produces a wide range of droplet sizes under all conditions, variation in orifice diameter has little effect on median droplet size. In addition, with this type of nozzle increases in airspeed and spray pressure result in relatively little decrease in median droplet diameter.

Factors Affecting Spray Distribution From an Aircraft

If maximum effectiveness is to be obtained from an aircraft application of an agricultural chemical, the application to the treatment area should be as uniform as possible while meeting the specific requirements of droplet size, foliage penetration, droplet coverage, etc. The fulfillment of this requirement necessitates the use of equipment yielding symmetrical single-swath patterns which, when properly overlapped, result in the required uniform over-all distribution (2, 9). Since the airflow about the airplane itself influences the spray distribution, the first problem then becomes that of placing nozzles or dispersal devices so that the desired pattern is obtained.

The effect of span-wise nozzle location.—Spray distribution measurements have shown that uniform and symmetrical spacing of spray nozzles does not yield a completely satisfactory single-swath pattern. Past experience has also shown that nozzle rearrangement to obtain a desired pattern is very difficult without an understanding of individual nozzle spray distribution and the factors affecting such distribution.

Rather extensive nozzle placement investigations have been conducted at the Forest Grove (Oregon) Station of the United States Department of Agriculture (19) and the Aircraft Research Center, A. and M. College of Texas (23, 31, 61, 62). The results, while not overlapping, show general similarity despite the differences in application equipment and evaluation techniques.

The general effect of variation in span-wise nozzle location is a progressive change in the nozzle distribution pattern and point of deposit as the nozzle is moved from the airplane center toward the wing tip; in general, the nozzle pattern tends to widen and become less concentrated. At some outer nozzle location this trend reverses, and the spray pattern may be deposited closer to the airplane center than patterns from some of the inner nozzles. This reversal is attributable to the proximity of the nozzle to the wing-tip vortex.

The outward flow of air under the wing causes the spray from the nozzles to move outward from the center. Spray from a nozzle on the aircraft center, however, moves toward the left (viewed from the rear). The twisting right-

to-left propeller slipstream affects the spray patterns from all nozzles located in it. The effect varies, depending upon the configuration of the airplane, but it usually results in a pulsating, erratic spray deposit with the spray from nozzles several feet to the right of the airplane center being moved toward the left. This effect is not so pronounced on the high-wing airplanes because of the damping effect of the fuselage.

The effect of airspeed.—Probably the most general concept of the effect of airspeed on a spray pattern is that with a particular nozzle and spray pressure an increased airspeed will result in a finer droplet spectrum from the nozzle. This is true as was shown previously in the discussion of the effect of airspeed on droplet size (59, 60). If this were the only effect of airspeed on spray distribution, however, then the widest swaths (no crosswind) could be obtained at the highest airspeeds; small droplets are more subject than large drops to the sidewash or sidewise movements of the air generated by the airplane. Actually, however, the intensity of the sidewash is partially dependent upon the airspeed of the airplane. More sidewash (and downwash) is obtained at the lower airspeeds.

To determine the effect of airspeed on distribution pattern several individual nozzles were tested at widely different airspeeds and the nozzle distribution compared (31). It was apparent that the widest swath would be obtained with the lowest airspeed. At an airspeed of 50 m.p.h the spray patterns from the two nozzles tested were both considerably wider than those at the two higher airspeeds. At this speed it would have been possible to obtain a total swath width of over 80 ft. with no nozzles beyond 9 ft. out on each side of the airplane. The average patterns obtained at the two higher speeds were quite similar, as would be expected, because the difference in their respective airspeeds was not great.

Although the airspeed affects the relative coarseness of the spray from a nozzle, it was apparent that the effect of the sidewise movement of the air around the airplane was paramount in changing the nozzle patterns and subsequent swath widths. From the results of these tests, it was concluded that the lowest practical airspeed would give the greatest swath width. Also, small variations in airspeed would not seriously change the distribution pattern obtained with a particular nozzle arrangement.

The effect of application height.—A series of tests was made to determine the degree of these changes at four different heights (3, 7, 12, and 15 ft. above the ground), heights that would encompass those used in actual practice for low level applications (31). In general, there was a change in the location of nozzle deposit and pattern that corroborated the general assumption that increased height results in increased swath width.

The change in spray pattern shape for each of the nozzles was not extreme between the successive heights. Between the lowest height and the highest, the nozzles on the right of the airplane showed little change in pattern shape or width. The nozzles on the left of the airplane showed, however, an increasingly wider pattern and a corresponding decrease in concentration with

the increasing height. This change in pattern shape probably can be attributed to the propeller effect which became more pronounced with an increase in height. Further, from a comparison of measured swath patterns it was apparent that an increase in height did not alter the pattern radically. The patterns were generally similar with the greatest change in pattern width occurring between the lower heights. In addition, pattern irregularities became less extreme with an increase in height.

The effect of orifice diameter.—In the previous discussion of factors affecting droplet size it was shown that at normal operating speeds and within certain limits orifice diameter did not affect the median droplet diameter. This was borne out by measured individual nozzle and swath patterns.

The measured spray distribution of several swath checks of the same nozzle arrangement made under similar conditions but using different size orifices ($\frac{1}{16}$ in. and $\frac{1}{8}$ in.) have been compared (31). The comparison showed that the volume of spray from the $\frac{1}{8}$ in. orifices was three times greater than that for the $\frac{1}{16}$ in. orifices, yet the shapes of the distribution patterns were almost identical. Although no swath measurements were made using $\frac{1}{32}$ in. orifices, it seems likely that the distribution would be similar, since the previous droplet analyses have shown little change in spray atomization at the 80 m.p.h. airspeed and the 35 p.s.i. spray pressure. It is not known to what extent the nozzle orifice diameter can be increased and still have these conditions of airspeed and spray pressure result in the same median droplet diameter. However, it is apparent that with a nozzle arrangement yielding an optimum distribution pattern, a wide range of application rates (gallon/acre) can be achieved by varying nozzle orifice size, if the airspeed is sufficiently high and the spray pressure is kept constant.

The effect of fluid atomization.—Spray pressure and spray fluid characteristics affect spray distribution indirectly inasmuch as they are determining factors in fluid atomization. In addition, airspeed, while affecting the airflow about the airplane, is also a vital factor in fluid atomization.

Information on the effect of atomization on airplane spray patterns for forest insect control has been presented by Isler & Thornton (24). In the investigation a comparison was made of the effects of three degrees of atomization (300, 150, and 80 μ m.m.d.) on deposit patterns of sprays released from a Stearman airplane flown at 50 ft. above the ground. The results showed that under the conditions of the tests the coarse atomization resulted in the narrowest swath, least uniform distribution across the swath, and excessively high deposit peaks. Although the fine spray gave a slightly wider and more uniform swath than the medium one, this small advantage was overshadowed by the higher loss of fine spray. It was concluded that a spray of medium atomization (150 m.m.d.) provides the most efficient swath pattern for forest spraying.

In a further effort to compare the effects of fluid atomization of spray distribution, pattern measurements have been made using water and Diesel oil as the spray fluids (61). The operating conditions being the same, the dif-

ference in fluid characteristics (surface tension, density, and viscosity) yielded a median droplet diameter of 320 μ for water and 210 μ for oil. The larger droplet size and greater density of water resulted in a spray pattern that was considerably narrower and more concentrated than that from oil.

While water is probably the most widely used spray carrier, it is not known at present to what extent the addition of emulsifiers, wetting agents, sticking agents, etc., to the usual spray solutions or emulsions will affect droplet size and pattern shapes.

The effect of variation in longitudinal distribution.—Chamberlin *et al.*(19) have found that considerable variation in deposit rate is encountered parallel to the flight path of the airplane. Their findings indicate considerable irregularity of deposit rate in successive foot-to-foot transects in the deposit area under the influence of the propeller slipstream, i.e., near the airplane center, and less irregularity at deposit points further outward on the distribution pattern.

At the Aircraft Research Center, A. and M. College of Texas (61), the longitudinal variation for one airplane using a pressure type spray system was found to be about 34 per cent above and 10 per cent below the mean deposit rate when the spray was sampled at successive 5 ft. intervals along the airplane's flight path. Although no definite frequency could be correlated with these variations, it seemed that there might have been the possibility that the irregularities occurred about every 20 ft. of forward travel of the airplane.

The effect of wind.—The effect of crosswind, which is probably the greatest and least controllable of the factors that influence spray distribution, complicates the achievement of a uniform-density spray application while operating under widely varying wind conditions.

Tests have been made that show the effect of crosswind on a spray pattern that was completely satisfactory with crosswinds near zero (61). With moderate crosswinds of 1 to 4 m.p.h. the pattern changed from a symmetrical, nearly uniform trapezoid to one which was narrower, irregular, and unsymmetrical with a pronounced hollow near the center. Left and right crosswinds resulted in extremely similar patterns with the exception that the left crosswind apparently counteracted the effect of the propeller slipstream to some extent and the center hollow was less pronounced with the moderate left crosswinds. Unfortunately, the general crosswind effect became pronounced even with the comparatively low crosswind conditions of 1 m.p.h. While the downwind side of the pattern was not substantially changed at very low crosswind velocities, the spray on the upwind side was more affected by the wind. The spray that would have been deposited beyond the upwind wing tip with no crosswind was deposited closer to center with a resultant narrowing and over-concentration of the pattern on this side.

The swath pattern dissymmetry and irregularity under the influence of crosswind had a serious and deleterious effect on over-all distribution, while the near-zero crosswind pattern gave reasonably good over-all distribution.

With a crosswind of only 2 m.p.h. the over-all distribution was seriously irregular, having some points of deposit 60 per cent below the average and others 55 per cent above. Increasing the overlap by decreasing the swath spacing from 50 ft. to 40 ft. did not improve the uniformity substantially because of the shape and dissymmetry of the pattern.

Additional tests were made at different wheel heights (1 to 2 ft., 5 ft., and 10 ft.) to determine the crosswind effect under these conditions; the results indicated that the patterns obtained at the various heights differed in degree but were generally similar. With a comparable crosswind the lateral displacement of the whole pattern increased with an increase in height as would be expected; but otherwise a similar distortion of the basic low-crosswind pattern was apparent at all heights. With an increase in crosswind above $\frac{1}{2}$ m.p.h., the pattern became unsymmetrical, with steep slopes and over-concentration on the upwind side and a pronounced hollow or underconcentration near the center. This indicated the probable futility of trying to achieve uniform over-all distribution under crosswind conditions by varying the height.

On the basis of the results of these tests to determine the effect of crosswind on spray patterns and with present dispersal equipment, which produces a wide range of droplet sizes, it seems unlikely that the most efficient spray distribution (over-all uniform application) can be achieved with a crosswind condition, particularly when such a crosswind has a velocity greater than 1 m.p.h. and, say, less than 8 to 10 m.p.h. It is felt that higher crosswinds would probably have a "smoothing out" effect; the greater crosswind would probably result in a wider, less concentrated triangular-type pattern with a long, sloping downwind side. With the increased width, multiple overlapping of adjacent swaths would probably minimize some of the irregularity in over-all distribution encountered at the low crosswinds.

To remedy the effect of crosswind, a spray composed of substantially uniform droplet sizes is needed. Then the drift characteristics of all the spray particles would be about the same. This would result in a displacement of the whole pattern with little distortion from the basic no-crosswind pattern.

Under present conditions an approach to the desired uniformity of droplet size can be obtained by limiting or decreasing the size of the largest droplets. This, however, is only a partial answer, because with the increase in fineness of the spray there is a proportionate increase in volume of the very small droplets which are almost wholly subject to uncontrolled drift.

Summary of factors affecting spray distribution from an aircraft.—Small variations of height, airspeed, orifice size, and spray pressure ordinarily do not radically alter a particular distribution pattern. Unfortunately, this is not the case with crosswind; not only is it an uncontrollable distribution factor but also, relatively small changes in velocity or direction can result in serious changes in the spray distribution pattern.

Drift.—While small-particle drift is important as far as potential hazard is concerned, it can result also in a substantial loss of spray material from the

treatment area. Little quantitative information is available on the relative loss of material in this manner, but some previous work (62) indicated that even with optimum conditions of low early morning temperature, high relative humidity (90 to 95 per cent), low height, and low wind velocity an average of only 75 per cent of the volume of water spray dispersed was collected. With less optimum conditions, such as those encountered in forest insect spraying, it has been estimated that on occasion as little as 10 per cent of the sprayed material might be collected on sampling devices. Actually, in a series of tests made under forest insect control conditions (24) it was shown that with three degrees of atomization—coarse (300 m.m.d.), medium (150 m.m.d.), and fine (80 m.m.d.)—the percentage recovery was in all cases less than 75 per cent. This loss must be attributed to a combination of several factors besides the drift of small particles, however. These other factors are evaporation and collection efficiency.

Evaporation.—At present, relatively little quantitative data are available on the evaporation losses of spray material while it is airborne. Certainly relative humidity and the volatility of the spray fluid are important factors; in addition, droplet size should affect evaporation rate because the descent velocity and the surface area of the droplets exposed to evaporation are both functions of droplet diameter.

Much remains to be done in the laboratory and in the field to correlate the factors affecting evaporation. Yeo & Thompson (63) have indicated that with a DDT solution of kerosene and Dieseline resulting in a spray spectrum of 5 to 250 (m.m.d. 70 to 80), 50 per cent of the solvent was lost in the few seconds required for the deposits to form.

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COTTON INSECTS AND THEIR CONTROL IN THE UNITED STATES[1]

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Cotton insects are responsible for an estimated annual loss of well over $200,000,000 to cotton planters, according to statistics released by various agencies. This tremendous loss is glaring evidence for the need of an adequate program of control if the planter is to realize a profit.

The cotton plant, with its many succulent leaves, nectaries on each leaf, and abundance of fruit is attractive to many species of insects, both injurious and beneficial. While the boll weevil is considered the most destructive species, the pink bollworm is potentially the more serious. Other insects including bollworms, leafworms, fleahopper, aphid, thrips, spider mites, plant bugs, and species of minor importance are responsible for crop losses in varying degrees.

The damage that the various insect pests and spider mites cause is not confined to the reduction in yield. Several species are responsible for reducing the quality of both lint and seed. It has been estimated that the loss in quality alone would more than pay for the cost of an adequate control program.

There has been a steady progress over the years in the economical control of these various injurious insects. Rainwater (1) reviewed briefly the development of controls during the period 1878 to 1951. In recent years, research entomologists have developed many new techniques, as well as new chemicals, for better control of these ever present cotton pests. Newly developed insecticides are first tested in the laboratory under controlled conditions; later these materials are placed in field tests for evaluation. Field tests are designed for statistical analyses of the infestation data and yields, in order to evaluate the insecticides or acaricides. Personnel of most of the Agricultural Experiment Stations and a number of Federal laboratories and field stations located in the Cotton Belt are continuously engaged in research toward the control of the various species of pests which attack cotton.

The species herein discussed are not listed in the order of their importance, but in the sequence of their usual occurrence during a cotton-growing season. The author reviewed this subject (2) in 1950 and the purpose of the present paper is to revise and bring the information up-to-date.

Thrips

As soon as cotton comes up to a stand in the spring, it is subject to attack by thrips. Several different species of thrips have been reported to cause a similar injury to cotton. Wardle & Simpson (3) found that thrips were responsible for premature and excessive defoliation but that the salivary secretion of the insect was not of a toxic nature. Gaines (4) and Watts (5) observed that thrips caused plants to become malformed and the small squares to be blasted.

A number of entomologists have reported (4, 6 to 12) the species of thrips which attack cotton in various sections of the Cotton Belt. The most common species reported in the southeastern states were: *Frankliniella fusca* (Hinds), *F. tritici* (Fitch), *F. runneri* (Morgan), and *Sericothrips variabilis* (Beach). In addition to several of these species, *Thrips tabaci* Lindeman and *F. exigua* Hood, were reported as being injurious in Texas. In New Mexico and California, *F. occidentalis* (Pergande) has been found to be injurious to cotton. It is apparent that a number of different species of thrips attain damaging populations throughout the Cotton Belt.

Eddy (13), Watts (5, 14, 15), Pfrimmer (12), and Newsom *et al.* (16) reported on the biology of several species of thrips in South Carolina, Texas, and Louisiana, respectively. In general, these studies indicated some difference in thrips life cycles which might be expected as a result of different environmental conditions.

The extent of damage to cotton produced by thrips varies in the different localities. Apparently, yields are reduced in areas where insufficient rainfall occurs during the latter part of the growing season. In areas where rainfall is abundant throughout the season, maturing of fruits is often delayed, yet maximum yields may be obtained in spite of damage caused by thrips early in the season. Gaines (4), Watts (5), and Dunnam & Clark (8) found that thrips not only reduced the number of bolls set, but also were responsible for delayed fruiting. Smith (17) found that thrips were responsible for plant injury which resulted in delayed fruiting. Fletcher *et al.* (18) found that both DDT and BHC were effective in reducing the thrips populations yet failed to increase the yields. Later, Gaines *et al.* (19, 20) reported on additional tests in which a number of insecticides proved effective against thrips, but the control of infestations failed to produce increases in yields. Most of the chlorinated hydrocarbon insecticides, as well as some of the phosphate insecticides, have proved effective for thrips control in many tests reported by various entomologists located throughout the Cotton Belt. In 1955, Am. Cyanamid 3911 (Thimet) was widely tested in fields of several states. Hanna (21) and others reported that this material used as a seed treatment gave good control of thrips, aphids, spider mites, and leaf miners for a period of four to six weeks, following plant emergence. Newsom *et al.* (16) reported that the control of thrips in Louisiana was not profitable.

Results of large acreage field tests in Texas reported by Ewing & Parencia (22) indicate that the control of insects attacking cotton only in the early season was profitable and that the cotton matured two to three weeks earlier

as a result of the control program. It was pointed out that the first of the three applications of early-season treatments was made primarily for thrips control; however, the subsequent applications were effective in controlling fleahoppers and overwintering boll weevils.

Owen (23, 24) reported that excellent profits were obtained in experiments designed to control thrips in West Texas, particularly when the infestation persisted after plants reached the fruiting stage, while Hanna (25) working in the eastern part of the State reported earlier fruiting for most years with significant gains in yields rarely demonstrated in small plat experiments. Kauffman & Stevenson (26) reported that during a three-year period, applications of insecticides for thrips control increased the yields considerably under Arizona conditions.

It is evident from the reports on thrips in the various states that infestations of this pest in most sections injure cotton by causing the plant to become malformed; however, chemical control of this pest has proved profitable only in certain areas. In those regions where control has proved profitable, thrips blast the small squares, thus causing a reduction in yield. It also appears that maximum benefits from thrips control are obtained where large areas are treated.

COTTON APHID

The cotton aphid, *Aphis gossypii* Glover, attacks seedling cotton, especially when the weather is cool and damp. This pest may retard growth of the plant and fruit production and, in cases of severe infestation, stands may be lost. Aphids may be controlled by the use of benzene hexachloride or parathion, but insecticidal control applied to seedling cotton rarely proves economical except in cases where losses in stand are prevented.

Aphid infestations occurring late in the season may cause premature defoliation and a decrease in yields. The honey dew secreted by the aphids affords an ideal medium for fungus growth which in turn may greatly reduce the quality of the lint. When calcium arsenate was used exclusively on large acreages for boll weevil control, it produced conditions favorable for aphid development, thus resulting in considerable damage. Gaines *et al.* (27) reported effective control of aphid infestations by the addition of nicotine to the calcium arsenate applied for boll weevil control.

In connection with tests conducted to control the boll weevil, entomologists in all the states report on the effectiveness of insecticides for aphid control. When the chlorinated hydrocarbon insecticides became available for wide use in the control of the weevil, the aphid problem was also greatly reduced. Benzene hexachloride was found to be effective against aphids as well as weevils. Several of the phosphate insecticides are also used to control cotton aphids.

COTTON FLEAHOPPER

The cotton fleahopper, *Psallus seriatus* (Reuter), also attacks cotton early in the season. Howard (28) first reported this insect as a pest of cotton in

1898. Later in 1924, Hunter (29) gave a brief account of severe injury to cotton in South Texas caused by the then-called "cotton flea." Reinhard (30, 31, 32) reported on the biology of the insect and suggested sulphur for its control. The injury to the cotton plant is characterized by an excessive blasting and shedding of small squares and a reduction in the number of fruiting branches which in turn results in either a tall whiplike growth of the main stem or in an increased number of vegetative branches. Several workers [Gaines (33); Reinhard (31); Hixon (34)] have reported on the host plant relationships. The fleahopper migrates from horsemint and other early spring weed host plants to cotton. When the cotton matures, the fleahopper then transfers to croton.

Ewing (35), Painter (36), King (37), and Brett *et al.* (38) investigated the possibility of the fleahopper being a vector of a plant disease. These workers concluded that any material injected into the plant by the insect did not spread far from the point of injury and that both the disturbance and shedding of squares were due to the multiplicity of insect bites. The fleahopper occurs throughout the Cotton Belt but perhaps causes the greatest damage in the Texas and Oklahoma areas. Ewing (39) and Ewing & McGarr (40) found that a mixture of Paris green or calcium arsenate and sulphur was more effective than sulphur alone in controlling this pest.

Most of the chlorinated hydrocarbon insecticides have been found to be highly effective against both the nymph and adult fleahopper. In areas where these insecticides are used to control insects in the early season, little injury has been reported from this pest. Parencia *et al.* (41), Parencia & Ewing (42), and Owen (23, 24) have demonstrated that chemical control of the fleahopper results in a profit to the planter.

BOLL WEEVIL

The boll weevil, *Anthonomus grandis* Boheman, crossed the Rio Grande in Brownsville, Texas, during 1892, and two years later it had spread through several counties in Southern Texas. Gradually, the weevil spread into other states and by 1922, it had covered practically the entire Cotton Belt from Texas to the East Coast. Research designed to control or eradicate the pest was begun in 1891 by an entomologist at the A. & M. College of Texas and in 1894 by workers in the United States Department of Agriculture. The work has been continued by the U.S.D.A. and the various state agricultural experiment stations in the cotton-growing area. Several workers [Hunter & Hinds (43); Hunter & Pierce (44); Fenton & Dunnam (45); Gaines (46)] have shown that there are two principal periods of dispersal and spread of this pest during the season. The first period occurs when the hibernating weevils leave winter quarters in search of food. The second period occurs later in the season and the time of its occurrence is dependent upon several factors: (*a*) intensity of weevil populations, (*b*) abundance of fruit, and (*c*) high percentage of infested fruit. The hibernating weevils migrate to cotton early in the season and feed on the young leaves and small

squares. When the squares have become one-third grown or larger, the female forms a cavity with the mouthparts in the square or boll in which an egg is deposited. The cavity is then sealed by secreting a muscilaginous substance from accessory glands of her female organs. The effect of the puncture and early feeding of the grub soon causes the square to flare and fall. Heavy weevil infestations result in serious boll injury, as well as square injury, caused by the grubs feeding on the contents of the fruit.

Losses from the boll weevil vary greatly from year to year. Low winter temperatures affect the survival and emergence of the adults the following spring. Weather conditions during the spring influence the development of the first broods and temperature and rainfall during the entire season are important factors affecting the rate of development of the pest.

Cultural practices are extremely important in the economical control of the boll weevil. Any control program should include all of the known practices which help to reduce the numbers of weevils. Several years ago, when the pink bollworm spread to the southern counties of Texas, strict regulations were imposed on the planters regarding the planting dates and early-fall destruction of stalks. The fall stalk destruction date was set sufficiently early to starve the boll weevil before it entered hibernation. During the years following fall destruction of stalks, the boll weevil infestation was greatly reduced and the need for chemical control was practically eliminated. Gaines & Johnston (47) reported the results of a fall stalk destruction program which was conducted on a voluntary basis in Williamson County, Texas, during 1947. The stalks were destroyed early by all planters in this county and the weevil infestations were greatly reduced the following year. Apparently a well executed fall plant destruction program is essential in the control program to reduce the losses caused by this pest.

During the last 50 years, various mechanical devices have been developed to destroy the boll weevil but none have proved profitable.

In 1909, Newell & Smith (48) recommended powdered arsenate of lead for the control of the weevil. Later, Coad (49) and Coad & Cassidy (50) reported that applications of calcium arsenate afforded good control of the weevil and caused no injury to the cotton plant. From the time of its discovery, calcium arsenate was recommended for the control of the weevil throughout the South. However, this insecticide produces conditions favorable to aphid development and is not effective against sucking type insects. An effort has been made by both manufacturers and entomologists to find a desirable substitute. After several years of experimentation, Young *et al.* (51) reported that a mixture of calcium arsenate containing 2 per cent nicotine in alternate applications with calcium arsenate effectively controlled the weevil and aphids. The limited supply of nicotine, however, prevented this program from being successful. The development of synthetic chlorinated hydrocarbon insecticides during and following World War II offered several possibilities. A number of entomologists [Becnel *et al.* (52); Dunnam & Calhoun (53); Ewing & Parencia (54, 55); Gaines & Dean (56, 57); Gaines

& Young (58); Ivy & Ewing (59); Ivy *et al.* (60); Parencia *et al.* (61); Rainwater & Bondy (62); Watts (63)] have shown that toxaphene or a mixture of benzene hexachloride and DDT was effective against the boll weevil, as well as most of the other cotton insects. Later, additional chemicals, including aldrin, dieldrin, heptachlor, and endrin, were manufactured and found to be effective for boll weevil control in various laboratories and field stations throughout the South.

Spraying cotton with arsenicals for weevil control has not proved profitable. However, results of tests conducted in several states in 1949 showed that the organic insecticides applied as spray emulsions at a low pressure and volume per acre were effective against the weevil. This method of insecticide application afforded many planters a better opportunity to more profitably control the weevil. It is estimated that currently more insecticides are applied as sprays than in dust form for weevil control. Economical boll weevil control can be obtained when either airplanes or ground machines are used to distribute the insecticides in either dust or spray form.

For several years, excellent control of the weevil was obtained with the new organic insecticides and planters practically stopped using the arsenicals. Some states withdrew calcium arsenate from the list of recommended materials. However, in 1952, weather conditions favorable for weevil development prevailed in the Lower Rio Grande Valley of Texas and planters experienced difficulty in securing adequate control of the weevil with organic insecticides. Wene (64) reported that dieldrin and endrin were the most effective of the materials used and suggested that the interval between applications should be reduced to three or four days during migration of the weevil. Good control was obtained the following years with both dieldrin and endrin, as well as other chlorinated hydrocarbons. Rainwater & Gaines (65) showed that certain organic insecticides were considerably less effective against the boll weevil when used late in the season than they were when used in the earlier stages of crop growth. Results of studies conducted by Gaines & Mistric (66) indicate that the food upon which the weevils develop may be partially responsible for any tolerance to insecticides which are applied later in the season.

Roussel & Clower (67) reported that the boll weevil became difficult to control with organic insecticides in certain areas of Louisiana during the late summer of 1954. The following year, planters in these areas experienced even more difficulty in obtaining economical control of the weevil with the commonly used insecticides. Tests conducted by the above listed entomologists indicated that the weevils in these areas exhibited a high degree of resistance to the chlorinated hydrocarbon insecticides.

Planters in certain other states also experienced some difficulty controlling weevils with organic insecticides during 1955, but positive evidence of resistance was not reported elsewhere. It is well known that failures to control weevils in some areas were attributable in part to weather conditions which were unusually favorable for weevil development. The growing season was rainy accompanied by high humidity.

In recent years, studies have been made in several states (68 to 72) to determine the effect on beneficial insect populations of insecticide applications made for the control of injurious insects on cotton. The general conclusion of these studies is that low dosages of insecticides used in the early-season control program have little effect on the beneficial insects while the higher dosages necessary to control the major pests later in the season greatly reduce the beneficial insect populations. In fact, after the second or third application of insecticide has been made in a boll weevil control program, the beneficial insects have been eliminated as a natural control factor.

The schedule of applications suggested for general use in the several states varies considerably. In some states, the early-season insect control program has proved profitable. In this program, insecticides are applied early in the season in order to reduce the overwintered population and prevent an early-season buildup in the cotton. In other states, results of experiments indicate that it is more profitable to delay insecticide applications until a certain degree of square infestation has developed. Entomologists in all cotton-growing states recommend that the insecticides be applied at least at five-day intervals in fields where critical square infestations exist.

BOLLWORMS

The bollworm, *Heliothis zea* (Boddie), occurs over the entire Cotton Belt as a pest of several cultivated crops. This insect overwinters in the pupal stage and the moth emerges early in the spring. The first brood of larvae feeds on legumes and corn, while the second brood attacks corn and sorghum crops, migrates to cotton fields and severely damages the more succulent cotton plants. Much information is available concerning host relationships, causes of outbreaks on cotton, and the control of this particular pest.

Riley (73) first studied the bollworm and recommended London purple and Paris green for its control. Later Quaintance & Brues (74) reported on its biology and control. They recommended arsenicals, the use of traps, and cultural practices to control the pest.

Considerable research has been directed toward obtaining information which would make it possible for entomologists to predict outbreaks of this pest. Thomas & Dunnam (75) showed that bollworm moths were attracted to succulent rank-growing cotton for the purpose of egg laying. Slow growing nonsucculent cotton was not attractive to the insect for oviposition. Lincoln & Isely (76) suggested that late-planted corn might be used as a trap crop to protect the cotton in Arkansas. Ewing & Ivy (77) found that more eggs and larger larval populations occurred on plats dusted with arsenicals than on undusted plats. These increases in bollworm infestations were associated with increased aphid populations which followed applications of calcium arsenate. Results of these tests indicated that more bollworm larvae survived when aphids were abundant than when no aphids were present and that this was apparently attributable to the predator preference for aphids. It is obvious that the factors involved here are complex, which makes it difficult to accurately predict outbreaks of the bollworm.

It is possible that insecticides used to control other harmful insects just prior to the appearance of the bollworm may kill off the beneficial insects and thus create an environment which is especially favorable for the bollworm to multiply. In areas where the bollworm is a damaging pest, it is suggested that insecticides should not be applied for at least a month prior to the normal occurrence of the pest in cotton. This procedure will allow normal populations of beneficial insects to develop and subsequently aid in reducing the numbers of bollworm eggs and the young larvae.

Calcium arsenate applied at the time when the worms were small proved effective in tests conducted by Moreland & Bibby (78), Gaines (79, 80), and Moreland et al. (81). However, planters experienced difficulty in obtaining satisfactory control with calcium arsenate, perhaps attributable in part to low dosages used, improper timing and the damaging aphid infestations, which developed following the use of the arsenical. Hanna & Gaines (82) found that a mixture of low-lime calcium arsenate, parathion, and DDT controlled the bollworm and prevented the development of aphids. By 1950 the planters preferred to use DDT and toxaphene for bollworm control, and the arsenical mixture was not generally used. Several research workers (83 to 90) concluded that toxaphene or DDT in either spray or dust form was effective for bollworm control. Applications of mixtures of benzene hexachloride-DDT and toxaphene-DDT also were found to give excellent control of the bollworm. Other chlorinated hydrocarbons which were used for boll weevil control did not prove effective in controlling the bollworm.

Later research disclosed that endrin was also a proficient insecticide for bollworm control. Toxaphene-DDT spray, DDT dust or spray, and toxaphene dust are the most popular insecticides used to control the bollworm at the present time.

The tobacco budworm, *Heliothis virescens* (Fabricius), since 1949 has come to be recognized as a pest of cotton. Brazzel et al. (91) compared the biology of the bollworm and tobacco budworm. This study disclosed that the larvae of these two species were similar in appearance and that both species probably attack cotton throughout a large portion of the Cotton Belt. In Louisiana, the tobacco budworm develops on winter legumes early in the spring and the following generations are largely dependent on cotton for food. They are usually more abundant in cotton during June and early July prior to the migration of the bollworm from corn to cotton. The insecticides recommended for control of the bollworm are likewise effective in preventing injury to cotton by the tobacco budworm.

PINK BOLLWORM

The pink bollworm, *Pectinophora gossypiella* (Saunders), is a worldwide pest of cotton and its feeding activities cause severe injury to the fruit. This pest is notably destructive because of its damage to the bolls and in many cases renders them unfit for picking. Cotton produced under heavily infested

conditions is always inferior in grade because of the presence of stains, is shorter in staple, and has less tensile strength.

The pink bollworm was first described from specimens collected in India in 1842, and it is believed to have been spread through infested seed shipments to Egypt in 1906. It was found in the Hawaiian Islands in 1909 and caused such severe damage that the planters were forced to abandon the production of cotton. According to Hunter (92, 93), the pink bollworm was introduced into Mexico in 1911 through shipments of cottonseed from Egypt. The first infestation in the United States was discovered in 1917; this, too, resulted from shipments of infested seed from the Laguna region of Mexico to Hearne, Texas. Later in that same year, additional infestations were found in several southeastern Texas counties. These infestations were eradicated by the use of noncotton or regulated zones or both, field clean-up, and other regulated restrictions. The following year an infestation was found in the Big Bend district of Texas. During a two-year period, cotton planting was abandoned in this area, but unfortunately when planting was resumed in 1921, the pink bollworm was still present.

Glick (94) made use of airplanes in a study relative to the pink bollworm migration and collected moths at altitudes as high as 3,000 feet. This information revealed that the Big Bend district could have been reinfested by moths migrating in from the Laguna in Mexico.

The biology of the pink bollworm was carefully studied in Mexico by Loftin *et al.* (95) and Ohlendorf (96). The persistence of infestation in the Big Bend district made it imperative to secure more complete information concerning the habits and biology of this pest. The Bureau of Entomology and Plant Quarantine in cooperation with the Texas Agricultural Experiment Station established a laboratory at Presidio in 1928 for the express purpose of studying the pink bollworm under local conditions. Much detailed information on the biology, host plants, dispersal, and hibernation of this pest was obtained, and a number of reports were published (97, 98, 99). After the pink bollworm became established in the Lower Rio Grande Valley in Texas and in Mexico, the research work under the direction of Federal workers was moved to Brownsville. Chapman & Lowry (100) reported infestations of larvae could be reduced with applications of fluorine compounds, but concluded that additional research was necessary to determine the usefulness of fluorine in a control program.

Before DDT was developed, little success in controlling the pink bollworm with chemicals had been attained. Robertson (101) reported success in controlling pink bollworm by use of DDT. The results of these tests indicated that the control obtained was attributable to killing the moths rather than the larvae. Tests conducted at Torreon, Mexico, by Chapman *et al.* (102) revealed that DDT would profitably control the pink bollworm.

Until recent years, quarantine and cultural control measures kept the pink bollworm confined primarily to irrigated areas of Texas adjacent to

Mexico. The expansion of the cotton acreage along the Rio Grande River in Texas and Mexico attended by at least three years of unfavorable weather conditions for established control measures allowed the pest to increase and spread over wide areas. During the last five years, the pink bollworm population has increased strikingly and spread over the entire state of Texas, practically all of Oklahoma, and to parts of Louisiana, Arkansas, New Mexico, Arizona, and into wild cotton in Florida.

Airplanes were again used by Glick (103) to collect insects in South Texas and pink bollworm moths were captured at altitudes up to 1,000 feet. These studies indicate that the rapid spread of this pest is a result of moth migration.

The destructiveness of the pink bollworm as a cotton pest is emphasized by the fact that prior to 1950, it caused negligible loss to the cotton crop in the United States, but in 1952 it was estimated to have caused a loss amounting to $28,000,000 in 38 South Texas counties. In 1952, when it became evident that the pink bollworm was spreading at an alarming rate, the research program was greatly expanded by the Entomology Research Branch, United States Department of Agriculture, in co-operation with several states.

During the period 1937 to 1954, the possibility of colonizing pink bollworm parasites in the United States was thoroughly explored. Hitherto, reports indicate that little promise is held for biological control.

The general recommendations for the control of the pink bollworm consist of (a) quarantine measures, (b) cultural control, and (c) chemical control. The quarantine regulations require that cotton, cotton products, and all articles associated with production and processing be so treated as to render them free of pink bollworms before they are moved to noninfested areas. The cultural control practices involve the proper planting time and the early destruction of the stalks by shredding and the plowing under of the crop residue. In the cooler, arid areas where lower temperatures prevail during the winter, the stalks should be left standing since the highest mortality occurs among the worms which overwinter in bolls on standing stalks. Where heavy infestations develop, losses may be reduced by the proper use of DDT. In areas where all of these practices can be employed, little damage results from this pest.

The present research program will, no doubt, yield much valuable information concerning this pest and should greatly improve the currently recommended control practices.

LEAFWORMS

The cotton leafworm, *Alabama argillacea* (Hubner), is one of the earliest known pests of cotton. This insect is not able to survive the winters in any part of the United States and infestations originate from flights of moths from Central or South America. Nevertheless, the leafworm is reported from some section of the Cotton Belt practically every year. The conditions affecting

the increase of this pest in its native habitat and the conditions existing in this country at the time the moths appear are the main factors governing its development.

The leafworm is primarily a leaf feeder. After the leaves have been destroyed, however, it may also devour the fruit. Since it prefers to feed upon the plant, the leafworm is easily controlled by the use of insecticides. Results of experiments conducted by Ivy & Scales (104) and Parencia *et al.* (105) indicated that practically all the chlorinated hydrocarbons used to control the major cotton insects, with the exception of DDT, proved effective in controlling the leafworm.

The cotton leafworm has not caused serious injury during the last few years, attributable, perhaps, to the thorough dusting and spraying programs which have been generally followed throughout the South Texas area. Unfavorable climatic conditions for the leafworm to develop in South and Central America may also have been a contributing factor.

The brown cotton leafworm, *Acontia dacia* Druce, was first observed attacking cotton in 1953. The following year it caused extensive damage in a few counties in Central Texas. Martin & Mistric (106) found that parathion, malathion, and endrin all were effective for the control of this pest. The damage caused by the brown leafworm first appears as small "buckshot" holes in the cotton leaves, which later become larger as the larvae continue to feed. This insect feeds principally upon the leaves and rarely attacks the fruit of cotton.

As a cotton pest, the brown leafworm has gradually spread to at least 20 counties in Central Texas, but the heaviest damage has been confined to a five-county area. In 1955, the small larvae attacked the young cotton and necessitated the use of malathion in the early-season control program.

Cabbage looper, *Trichoplusia ni* (Hubner), and related species have been reported damaging cotton in certain areas. The looper is principally a leaf feeder and was reported to have caused widespread damage to cotton in West Texas during 1955. In general, the chlorinated hydrocarbons used to control other insects will prevent injurious looper infestations. This is true only in incipient infestations. After the worms become larger, they are more difficult to kill. Endrin has been found to be the most effective insecticide for looper control up to the present time.

The salt-marsh caterpillar, *Estigmene acrea* (Drury), has been reported to cause extensive injury to cotton in the western part of the Cotton Belt. Either dusts or sprays containing toxaphene-DDT, parathion, and endrin have been found to be effective for the control of this pest.

The yellow-striped armyworm, *Prodenia ornithogalli* Guenee, feeds on the leaves of cotton early in the growing season, but later it may also attack the fruit. Toxaphene, DDT, and dieldrin have been found effective for the control of this pest. Another species, *P. praefica* Grote, the western yellow-striped armyworm, has been reported to damage cotton in California. Toxaphene or DDT has been used with success in the control of this insect.

There are several species of moths of the genus *Anomis* which attack cotton in different countries. All of these species are principally leaf feeders and may be mistaken for the cotton leafworm. Three species, *A. erosa* Hubner, *A. flava fimbriago* Stephens, and *A. texana* Riley, have been reported to damage cotton in this country. However, the occasional infestations of these species have not afforded an opportunity to entomologists for the development of control measures.

The garden webworm, *Loxostege similalis* (Guenèe), also may be found attacking cotton leaves. Most of the chlorinated hydrocarbon insecticides used to control other pests on cotton also have been found effective against this pest, provided applications for control are made when the worms are small and before webbing becomes extensive.

Spider Mites

Infestations of spider mites develop on cotton throughout the Cotton Belt. These pests attack the underside of leaves and cause a discoloration and subsequent defoliation of the plants. Roussel *et al.* (107) reported that spider mite injury to cotton resulted in a 45 per cent reduction in the amount of seed cotton produced. In addition, there were reductions in the number of seeds per boll and in the weight, viability, and oil content of the seed.

Worsham (108) and McGregor & McDonough (109) reported on the biology of the spider mite, *Tetranychus bimaculatus* Harvey, now considered *T. telarius* (Linnaeus), and discussed the damage it produces to cotton. Later, McGregor (110) suggested the use of lime-sulphur and kerosene emulsion for its control.

In more recent years, at least a dozen species of spider mites have been reported as being injurious to cotton. The two-spotted spider mite, *T. telarius*, occurs throughout the Cotton Belt and is perhaps the most difficult to control. The strawberry spider mite, *Tetranychus atlanticus* McGregor, and the Pacific mite, *Tetranychus pacificus* McGregor, occur in the western part of the Cotton Belt, according to Baker & Pritchard (111). In Texas and Louisiana, both *Tetranychus desertorum* Banks and *Tetranychus tumidus* Banks may be found on cotton along with the two-spotted spider mite. It is generally believed that the use of the chlorinated hydrocarbons for cotton insect control will create an environment favorable to spider mite increases. The addition of sulphur to insecticidal dusts prevented the increase of certain species of mites but it did not prevent the development of all species.

Isely (112) showed that a DN-sulphur dust mixture was effective in controlling spider mites in Arkansas. He indicated, however, that the method of application was the most important factor in the control program. Iglinsky & Gaines (113) reported that sulphur and parathion were effective in controlling *Septanychus* sp. now known to be *T. desertorum* Banks. Iglinsky (114) compared the life histories of *T. telarius* (*bimaculatus*) and *Tetranychus opuntiae* Banks now considered the same as *T. desertorum*. He also studied the effect of several phosphorus insecticides on *T. desertorum*. Magee &

Gaines (115) tested several new phosphorus compounds and Gaines *et al.* (116) made laboratory studies of the toxicity of 14 phosphorus and sulphur compounds for control of the desert spider mite. Several were found to be highly effective. Young & Gaines (117) found that applications of a mixture of calcium arsenate and parathion resulted in satisfactory control of the boll weevil, aphids, and the two-spotted mite.

Ivy *et al.* (118), Iglinsky (114), Tsi (119), and Ivy (120) found the systemic insecticide schradan effective in controlling aphids and spider mites when used as either a foliage spray, seed treatment, or when placed in nutrient solution in which seedling cotton plants were growing. Gaines *et al.* (121) reported that demeton applied to cotton in the field as a foliage spray was effective in spider mite control for a period of three to four weeks.

In the western part of the Cotton Belt, demeton and parathion have been used extensively for the control of all species of spider mites. Aramite was found to be effective and has been used commercially; however, the high dosage of this material necessary to control certain species has limited its use. Sulphur has been used extensively for the control of certain species of spider mites and is incorporated in chlorinated hydrocarbon insecticidal dusts in order to control some species and for the suppression of others. This mixture has not always proved effective in preventing a mite infestation. Since sulphur cannot be incorporated into sprays, it is necessary to use other materials which are more expensive.

HEMIPTEROUS INSECTS

There are a number of hemipterous insects or true bugs which attack cotton, especially in the western areas of the Cotton Belt. As early as 1910, Morrill (122) reported that several species of hemipterous bugs had caused injury to cotton. Later he (123) described the damage resulting from infestations of *Lygus* spp. and the southwestern cotton stainer, *Dysdercus albidibentris* Stål. He stated that the *Lygus* bugs transferred from alfalfa to cotton and attacked the squares, thus causing them to shed. The stainer destroyed young bolls and attacked the mature bolls, which resulted in the lint being stained. McGregor (124) studied the biology and damage caused by *Lygus elisus* Van Duzee and recommended sulphur for its control.

Cassidy & Barber (125) reported that the most injurious species of plant bugs in Arizona were as follows: *Lygus hesperus* Knight, *L. pratensis oblineatus* (Say), and *Creontiades femoralis* Van Duzee. They considered *Euschistus impictiventris* Stål, *Chlorochroa sayi* Stål, and *Thyanta custator* (Fabricius) the most destructive stinkbugs. The cotton stainer, *D. mimulus* Hussey, also was reported to cause damage in Arizona. These workers concluded that hemipterous insects reduce the total amount of the crop by causing the plants to shed fruit and to become malformed. The boll-feeding species reduce the grade and value of lint because of staining. Mixtures of calcium arsenate-Paris green and Paris green-sulphur were effective in controlling these insects and excellent profits were realized by their use.

Work conducted later in Arizona by Stevenson & Kauffman (126) proved the organic insecticides to be more effective in controlling hemipterous insects than the arsenical-sulphur mixture. Either DDT-sulphur or benzene hexachloride was effective in controlling the species in this group. Indications were, however, that DDT-sulphur was the most satisfactory insecticide for general use against cotton insects in Arizona.

Owen & Gaines (127) found that benzene hexachloride, DDT-benzene hexachloride, DDT, or toxaphene dusts all proved equally effective for *L. oblineatus* (Say) control in western Texas. Later Owen (128) and Noble (129) found that the management of alfalfa crops in that area affected the extent of *Lygus* infestations in nearby cotton fields. *Lygus* populations in Texas were never numerous in alfalfa which was cut for hay; on the other hand, the population developed to sizable proportions if the alfalfa was allowed to produce seed. When the alfalfa matured, the bugs migrated to cotton fields reaching some two miles from the alfalfa plantings. Results of tests showed that *Lygus* bugs were controlled, and excellent profits were realized by the use of one or two properly timed applications of insecticides.

PESTS OF MINOR IMPORTANCE

A number of species of insects cause what is considered minor damage to cotton each year. It is possible that these species may develop to damaging numbers in a local area.

The cotton square borer, *Strymon melinus* (Hubner) causes some injury to cotton every year in the Southwest. The larvae destroy the squares according to Reinhard (130). The organic insecticides used to control the major cotton insects have proved effective for the control of the square borer.

The greenhouse leaf tier, *Udea rubigalis* (Guenèe), has been reported damaging to cotton in the San Joaquin Valley of California. Either DDT, toxaphene, or endrin is effective in the control of this pest.

Leafhoppers are commonly found in cotton fields throughout the Cotton Belt, but only in California has injury to cotton been reported. *Empoasca solana* DeLong and *E. fabae* (Harris) are reported to be phloem feeders on some crops and cause damage to cotton typical of this type of feeding. H. T. Reynolds (unpublished) reported satisfactory control of *E. solana* with parathion, demeton, or diazinon.

Some species of leaf rollers have been reported to attack cotton. *Platynota stultana* (Walshingham) has been known to cause extensive damage in California, Arizona, and New Mexico. Atkins *et al.* (131) reported DDT, parathion, methyl parathion, or Perthane to be effective in the control of this pest.

During the past three drouth years, the serpentine leaf miner, *Liriomyza subpusilla* (Meigen) has been reported in large numbers, causing injury to cotton early in the season in certain areas of Texas. Parathion has proved effective in reducing the numbers of this pest.

Several species of mirids [Faulkner (132)], other than the *Lygus* spp., are known to injure cotton. They produce damage similar to that caused by *Lygus* spp. Any one species or several species in this group may increase to damaging numbers in a given locality in any year, thus making it necessary to use control measures. A species of the cotton stainer group, *D. suturellus* (Herrich-Schaeffer), has been reported from Florida and *D. obscuratus* Distant from Texas.

Wireworms, false wireworms, and the seed-corn maggot all have been reported to prevent the establishment of a stand. Aldrin, dieldrin, heptachlor, or lindane used as a seed treatment has proved effective in preventing damage from these soil pests. Several species of root aphids are known to attack cotton in an area near the East Coast. During the 1955 season, three species of Collembola were associated with poor stands of cotton in some areas of West Texas.

The fall armyworm, *Laphygma frugiperda* (J. E. Smith), and *L. exigua* (Hübner) are known to cause injury to cotton. The larvae are nocturnal feeders and may occur in sufficient numbers to defoliate the plants. Aldrin, dieldrin, endrin, benzene hexachloride, toxaphene, and DDT all have been found effective against these pests. Several species of cutworms are known to attack seedling cotton. Toxaphene, DDT, dieldrin and endrin are effective for most of these species.

The cotton leaf perforator, *Bucculatrix thurberiella* Busck, at times has been responsible for the defoliation of cotton in California and Arizona. This insect is also known to occur in Texas but has not been reported to cause serious damage. Several insecticides including DDT, toxaphene, endrin, and parathion have proved effective in controlling this species.

Whiteflies have been recorded in injurious numbers in the western part of the Cotton Belt. Smith (17) stated that three species had been collected from cotton but that *Trialeurodes pergandei* (Quaintance) was perhaps the most important species. The early destruction of weed host plants growing adjacent to cotton fields will reduce the numbers of this insect which later migrate to cotton.

General Recommendations for Chemical Control

It is the responsibility of entomologists of extension services and experiment stations to prepare recommendations for the control of cotton insects in their respective states. To assist the entomologists in this undertaking, a conference of State and Federal workers concerned with cotton insect control is held each year. The purpose of this conference is to review the results of all research work conducted the previous year and to use the knowledge gained as a guide for the recommendations the following year. A report of this conference is prepared and made available to all conferees as well as anyone else interested in cotton insect control. The table prepared by the conferees in the fall of 1955 is herein reproduced, in order to show the general recommendations for cotton insect control used throughout the Cotton

TABLE I

RECOMMENDED DOSAGES FOR THE PRINCIPAL INSECTICIDES AND MITICIDES USED FOR THE CONTROL OF CERTAIN COTTON PESTS (POUNDS PER ACRE OF TECHNICAL MATERIAL IN A DUST OR EMULSION SPRAY)

Pesticide	Boll weevil	Boll-worm	Cotton aphid	Cotton flea-hopper	Cotton leaf-worm	Cut worms	Fall army-worm	Grass-hoppers	Lygus and other mirids	Pink boll-worm	Spider mites	Stink bugs	Thrips
Aldrin	0.25-0.75						0.25-0.5	0.10-0.25	0.25-0.75				0.08-0.15
Aramite											0.33-1.0		
BHC (gamma)	0.30-0.45		0.3-0.6	0.1			0.4-0.6	0.3-0.5	0.30-0.45			0.5	0.1-0.2
Calcium arsenate*	7-10	12-15			7-10								
Chlordane	1.0-1.5		0.25-0.5	0.2			1.5-2.0	0.5-1.5	1.0-1.5				0.5-1.0
Chlorthion	0.30-1.0				0.25-0.5				0.30-1.0		0.25-0.5‡		0.375
DDT		0.5-1.5		0.5		1-2‡	0.5-1.0		1.0-1.5	2-3			0.25-1.50
Demeton†			0.125-0.4								0.125-0.4		
Dieldrin	0.15-0.50			0.1		0.3-0.5	0.2-0.3	0.07-0.125	0.15-0.50			0.5	0.05-0.15
Endrin	0.2-0.5	0.2-0.5		0.08-0.15	0.2-0.5	0.2-0.5	0.2-0.3	0.2-0.5	0.2-0.5				0.08-0.15
Heptachlor	0.25-0.75			0.2				0.25-0.50	0.25-0.75			1.0	0.08-0.15
Malathion			0.4-0.75		0.25-0.5						0.25-0.75‡		
Methyl parathion	0.25-0.5		0.25-0.5	0.2	0.25-0.5						0.25-0.5‡		
Parathion			0.1-0.25		0.125						0.1-0.4‡		
Sulfur*											20-60‡		
Toxaphene	2-3	2-4		0.75-1.0	1.5-2.0	2-4	2-2.5	1.0-2.5	2-3			6.0	0.75-1.0

* Dust only.
† Spray only.
‡ Does not control all species.

Belt. Detailed recommendations vary with the requirements of the region or locality. (See Table I.)

The development of the synthetic organic insecticides which can be formulated into emulsifiable concentrates has made it possible to control cotton pests with sprays. The trend in the use of insecticides is toward low-volume sprays which have proved to be as effective as dusts. Sprays may be applied either by ground machines or by airplanes and under weather conditions which would render dusts ineffective.

Surveys conducted on a cooperative basis by State and Federal agencies in many of the states in the Cotton Belt have developed an excellent advisory service to the planters, as well as to industry. As a result of this service, planters are informed of the insect situation through radio and press at regular intervals throughout the season and this information serves to direct insecticides to areas where critical need for their use may occur.

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INSECTICIDAL CONTROL OF THE
SPREAD OF PLANT VIRUSES[1]

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As most common virus diseases of crops are spread by insects, their control might seem to raise no particular difficulties; there is now a range of insecticides that kill most of the insects that transmit viruses, and the efficient use of these could a priori be expected to prevent or at any rate greatly decrease the spread of viruses. Contrary to this expectation, however, applying insecticides to crops has, more often than not, failed to decrease the incidence of virus diseases in the sprayed crops and has sometimes actually increased it, even though field inspections of the crops indicated that the insecticide has "controlled" the specific insect vector. Most early attempts to control virus diseases by spraying failed and, although there are now results that indicate success against a few diseases, many still remain uncontrollable by this method.

As a preliminary to the main subject, a few words must be said about the differences between controlling insects as direct pests and as vectors of viruses. In turn this will call for some consideration of the way the incidence of virus disease increases in a crop and of the behaviour of insects in transmitting different viruses. The direct damage done to a crop by an insect will be roughly proportional to the number of insects that infest it multiplied by the length of time the insects feed on it. This is, of course, only very roughly so, for the condition of the plants when they become infested will also affect the damage caused by a given amount of feeding, but in general the losses will be decreased as the total number of "insect days" on the crop is decreased. In contrast, the spread of viruses is not proportional to the extent or duration of insect infestation of a crop. Three conditions must be fulfilled for an insect-transmitted virus to spread. First, there must be a source of the virus; secondly, the insect vector must be present, and, thirdly, the insect must move about. Quantitative differences in any one of these conditions will affect the extent to which a virus disease increases in a crop.

Aphids cause damage as pests mainly because of the large infestations of wingless individuals that are produced when conditions are suitable. But apterae of many species live mostly on the plants where they are bred, and so are relatively unimportant as vectors of viruses compared with the winged forms that are responsible for starting the infestation or that are produced within the crop. Hence the problem of controlling aphids as pests is mainly the problem of preventing the wingless forms from multiplying to damaging numbers, whereas the problem of controlling virus diseases is to kill the winged forms before they infect healthy plants. Sometimes viruses may be

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[1] The survey of the literature pertaining to this review was completed in February, 1956.

spread extensively in a crop by winged aphids that enter it but that do not stay and breed and so never lead to an infestation of wingless ones [Dickson *et al.* (1)].

A crop that starts its life virus-free, as do most that are raised annually from true seeds, will remain free from viruses unless these are brought into it from outside. Once virus-infected plants occur in a crop, however, there are two ways in which the number of infected plants can be increased, either by spread from those already infected or by more infective insects entering the crop. Obviously, a treatment that prevented one type of spread might be ineffective against another.

The behaviour of insects in transmitting different viruses differs in ways that might be expected to affect the value of insecticides in decreasing spread. We can conveniently follow the terminology of Watson & Roberts (2) and call viruses "persistent" or "nonpersistent," depending on whether the vectors, once they have become able to infect healthy plants, remain able to do this for long periods or lose the ability within hours. It is characteristic of the persistent viruses that insects do not become able to infect a healthy plant immediately after they have acquired virus from an infected one. The period between the two processes varies from a few hours with some viruses to many days with others. The viruses are imbibed with the plant sap, pass through the gut wall into the haemocoel, thence to the salivary glands, from which they can be injected into uninfected plants. Many of the persistent viruses seem to occur predominantly in the phloem and seem to have to be placed in the phloem of a healthy plant if infection is to occur: hence, for insects to acquire virus and become infective, or for infective insects to infect healthy plants, they have to be on plants at least long enough for them to feed on the phloem. By contrast many of the nonpersistent viruses seem to occur predominantly in the epidermal cells of infected plants [Bawden, Hamlyn & Watson (3)], from which they can be obtained by aphids in feeding periods of a minute or less. Infective insects can also infect healthy plants during equally brief feeding periods; the whole process of acquiring virus and infecting a healthy plant can occur within a few minutes [Watson (4)] or less [Sylvester (73)], and the viruses seem to be carried in the mouth-parts of the vectors [Bradley & Ganong (5)]. Insects that have not fed for some time immediately before they feed for a brief period on an infected plant are much more likely to become infective than are insects that have fed continuously [Watson (4)], a fact that contributes to the importance of winged forms as vectors of this type of virus, even though in experiments all forms of insects, whether adults or larvae, winged or wingless, are usually found to be equally good vectors.

Obviously from this brief survey the chances of any insecticide being useful against virus diseases can be expected to vary with different viruses, depending on whether the virus is of the persistent or nonpersistent type and whether spread is mainly from infected to healthy plants within a crop or whether spread mainly occurs by virus being brought into the crop by

already infective insects. To prevent the spread of nonpersistent viruses, an insecticide would have to kill incoming winged insects very quickly, whereas a slower acting insecticide could be expected to prevent the spread of persistent viruses, particularly spread from plant to plant within a crop. The experiments reviewed below show that this expectation is, in general, realized. Many of the experiments, particularly of the earlier ones, were empirical trials, made without a detailed knowledge of the sources of infection or of the manner in which vectors transmitted. However, had this knowledge existed and the early trials been restricted to the control of persistent viruses that mainly spread within a crop, they would still probably have failed, for the insecticides then in use, although effective in killing insects already infesting a crop, did no more than this. Success in controlling virus diseases by insecticides has depended, more than on anything else, on the development of insecticides that remain active, either on or in the sprayed plants, for days or weeks after they are applied. Typical examples are DDT, parathion and the systemic organo-phosphorus compounds, which can be applied before insects infest the crops and kill them when they do.

EARLY EXPERIMENTS

This is not a historical review, but it will be worth examining some of the early attempts at control to see why they failed. Samuel, Bald & Pittman (6) tried unsuccessfully to control spotted wilt of tomatoes in Australia by dusting fortnightly for two months with sulphur, sulphur and nicotine, or a mixture of copper carbonate, lead arsenate, sulphur, and nicotine dusts. Later attempts with nicotine dust or potassium sulphide sprays also failed for the same reason, namely because the insecticides killed the vectors, thrips, on the crop but did not affect those that came into the crop between spraying [Bald (7)]. Moore (8) was successful by spraying tomato plants with tartar emetic and sugar, a lethal bait for thrips, and decreased the incidence of spotted wilt from 100 per cent in unsprayed plots to 5 per cent in the sprayed plots. Magee, Morgan & Johnston (9), with a similar spray applied twice weekly, reduced spotted wilt from 27 per cent in unsprayed plots to 9 per cent in sprayed.

Many attempts were made to decrease virus spread in potato crops by spraying or dusting with nicotine, rotenone, or pyrethrum formulations to kill the aphid vectors. Cleveland (10) attempted to control leaf roll by spraying small plots containing one row of infected plants with a mixture of lead arsenate, nicotine sulphate, and Bordeaux mixture. Leaf roll incidence was reduced from 81 per cent in an unsprayed plot to 31 per cent in a plot sprayed seven times. As the leafhopper populations were decreased more than those of other insects, Cleveland wrongly concluded that they were probably the vectors. From field work of this type, however, it is rarely possible to identify vectors. Although workers have often assumed that the insect present in largest numbers is the vector of a virus that spreads extensively, the assumption is rarely justified, for a virus may infect the

majority of plants in a crop but still be spread by a species that never becomes numerous enough to constitute a serious pest. Similarly, yield trials alone are of little value in assessing the value of sprays in controlling virus diseases, because the sprays will affect losses caused by pests that are not vectors. For instance, Klapp (11) found that nicotine sprays greatly increased yields of potatoes by decreasing injury by aphids, but even weekly applications failed to stop virus spread. Fenjves (12) also found that nicotine gave no protection against incoming infective winged aphids, and even spraying eight times or dusting six, beginning in early June, only slightly reduced leaf roll spread. Doncaster & Gregory (13) fumigated with nicotine in mid-July in England and killed almost all the aphids, but this had little effect on virus incidence, as most of the spread had already occurred.

Intensive work on potato aphid control has been done in Maine since 1940, but early efforts to protect the plants from virus introduced by incoming aphids failed [Bronson (14)]. Areas treated three or four times with derris dusts or sprays outyielded check areas by as much as a quarter, but more of the seed tubers saved from the treated stock had leaf roll than from the untreated; preventing the damage caused by the very large aphid populations on the unsprayed plots prolonged the life of the sprayed plants and exposed them to incoming infective aphids late in the season.

Some early attempts to stop the spread of other aphid-borne viruses were partially successful. Crumb & McWhorter (15) planted beans next to a field of red clover infected with yellow bean mosaic, which was carried to the beans by migrating *Acyrthosiphon pisum* (Harris) [*Macrosiphum pisi* (Harris)], although the aphids did not colonize the beans. Dusting the beans weekly with nicotine halved the incidence of disease, but this was not an economic return. By dusting peas five times at six-day intervals with cubé powder, even though only single rows were treated, Huckett (16) decreased the viruses spreading from alfalfa and clover and got an economic increase in yield. The third dusting had the greatest effect, probably because it caught most of the alatae soon after they had arrived and prevented aphids from developing and spreading virus within the crop.

Lettuce yellows virus (aster yellows), transmitted by the leafhopper *Macrosteles fascifrons* (Stål) [*M. divisus* (Uhler)], limited lettuce growing in New Jersey, and many attempts were made to control it. Pepper & Haenseler (17) found that derris and pyrethrum, applied to small plots five times at ten-day intervals, prevented leafhopper infestation but had little effect on yellows. They thought that the leafhoppers moved from plot to plot throughout the season, and when larger plots of half-an-acre were dusted every week on seven occasions, not only was the leafhopper population decreased by 96 per cent, but the incidence of yellows was also decreased from 77 to 11 per cent. Linn (18) also found that three dustings with pyrethrum-sulphur or derris gave satisfactory control of yellows in lettuce and endive crops, the best treatment reducing its incidence from 49 per cent in untreated plots to 9 per cent.

Curly top virus of beet has caused great losses in the arid southwest of the United States and from 1931 onwards sugar beet companies in California financed a large-scale control programme against the vector, *Circulifer tenellus* (Baker). This was probably the first attempt to control vectors away from the crops it was desired to protect, for the spray (pyrethrum in diesel oil) was applied in the foothill-desert areas where the insects congregate in the autumn and winter, and on Russian thistle, their chief summer host. The hoppers carry the curly top virus from wild hosts to beet, tomatoes, spinach, beans, cucurbits, and other crops on which they feed while migrating in the spring. Cook (19) attempted to evaluate the control programme but this was difficult without any unsprayed areas as controls. The data were inadequate for assessing the potential damage in the absence of spraying.

Douglass, Wakeland & Gillett (20) tried to control curly top by spraying beet crops with nicotine, derris, or pyrethrum four times during June. Only pyrethrum checked the leafhopper, but even this did not control curly top. The spring migration extends over a period of two to five weeks in different years, at a time when the beet are seedlings and very susceptible. The insecticides failed because, although they killed insects present on the crops when they were applied, they did not prevent later infestation. Romney (21) was more successful in preventing the leafhoppers from infecting beet seed plants in Arizona and New Mexico during the autumn. Pyrethrum in oil satisfactorily controlled the insects on the crop, and the incidence of curly top in the following spring was reduced, often by as much as a half of that in the checks, which resulted in a big increase in yield. Once the plants were big enough to cover the soil, the microclimate was unsatisfactory for leafhoppers, so it was necessary to control them only during the early period of growth.

It may be concluded from these early attempts to control the spread of viruses by using such insecticides as nicotine, derris, and pyrethrum, that it is not sufficient to kill the insects on the crop. The insecticides did not remain active on the foliage, and very frequent applications were necessary to affect virus spread. These were expensive and often failed because incoming insects introduced virus or spread it from plant to plant in the intervals between applications.

EXPERIMENTS WITH CONTACT PERSISTENT INSECTICIDES

The situation changed radically with the introduction of new types of insecticides during the 1940's, for it seemed that DDT and other synthetic products would protect plants from insects for considerable periods and so could reasonably be expected to decrease the spread of viruses.

Leafhoppers.—Douglass, Gibson & Hallcock (22) planted a variety of beet susceptible to curly top in conditions where leafhopper infestations were to be expected and dusted the crop with DDT 12 times at three to nine day intervals during the spring migration. No plots were left unsprayed, but leafhoppers were more numerous than during the previous 10 years, and

almost all unsprayed tomatoes in fields nearby were killed with curly top, but only 37 per cent of the beet plants became infected and few died; they yielded better than resistant varieties nearby, but the total dose of DDT, 27 lb. per acre, was too high for general practice.

Further attempts were made to control the leafhoppers on their Californian desert hosts by applying DDT in diesel oil three times during the winter and spring, as an aerosol, from aeroplane or ground machines [Armitage (23)]. The five-year average (to 1950) of curly top incidence in tomatoes was 28 per cent; it was 44 per cent in 1950 but was reduced to 5 per cent in the San Joaquin Valley in 1951 after the first season of large-scale spraying. It should be emphasized, however, that it is not possible to evaluate this chemical control program because of the absence of comparable untreated areas.

Douglass, Romney & Jones (24) also attacked the leafhoppers in their breeding grounds in Idaho, spraying soon after the nymphs had hatched because these are more susceptible than the adults to DDT emulsion. After spraying 15,000 and 11,350 acres of the most heavily infested breeding areas in 1950 and 1953, they estimated that curly top incidence was reduced in 1953 from an expected 11 per cent to 1.9 per cent in snap beans. The results of Murphy & Douglass (25) suggest that, except for planting resistant varieties, this indirect method of attack is the only satisfactory way of controlling curly top. They planted four varieties of beet with different degrees of susceptibility to curly top and sprayed them four times with DDT emulsion at weekly intervals. Most of the leafhoppers in the crop were killed, but when many insects came into the suceptible varieties DDT did not protect them enough to produce a commercial crop.

Ashdown & Watkins (26) found that DDT dusts were more effective than rotenone plus sulphur or pyrethrum plus sulphur for lettuce yellows control, but dusting of large plots every five days after germination to within three weeks of harvest only decreased the proportion of plants with yellows from 80 to 30 per cent.

Hoffman (27, 28) sprayed lettuce with parathion in June and July and the edges of the field, where infective leafhoppers came from, with parathion, DDT, or both, from May to August. More than half the lettuce was infected with aster yellows virus in a nearby field with no border control, in contrast to 7 per cent (sprayed) and 11 per cent (unsprayed) in the field with border control. Spraying the borders was more effective than spraying the crop.

Aster yellows virus similarly affects carrot crops. DDT prevented the vector, *M. fascifrons*, from breeding in the carrots, but when small eight-row plots were used virus was spread from unsprayed to sprayed plots. In half or three-quarter-acre plots, three applications reduced yellows from 40 to 18 per cent using DDT dust and to 8 per cent using DDT wettable powder plus oil emulsion [Hervey & Schroeder (29)]. The results with DDT are no better than those obtained with pyrethrum or derris (16, 17), and it has

not proved possible to prevent incoming leafhoppers from introducing the virus, though subsequent spread within the crop can be prevented.

Thrips.—Costa, Forster & Fraga (30) and Fraga & Costa (31) sprayed tomatoes every three to six days, for seven weeks after transplanting, with a parathion compound, toxaphene, and tartar emetic. The plots were small (3 rows of 10 plants), but some treatments reduced the incidence of spotted wilt. DDT dusts applied fortnightly gave good thrips control, but only reduced spotted wilt incidence slightly because most of the infection was caused by incoming infective thrips [Michelbacher *et al.* (32)]. However, DDT emulsion applied once, twice, and thrice per week reduced infection from 74 per cent in nearby untreated tomatoes to 28, 15, and 10.5 per cent respectively. The formulation of an insecticide may affect the speed with which it kills or paralyses, and so its effectiveness for stopping virus spread.

Viruses transmitted by thrips are of the persistent type; the insects do not become infective immediately after they have fed on a diseased plant. Unfortunately the sources of these viruses seem to be mainly weeds, and although preventing spread within the crop is not too difficult, none of the older or newer insecticides have prevented the introduction of virus from outside.

Aphids.—Most aphid-transmitted viruses are of the "non-persistent" type, and few attempts have been made recently to stop their spread by insecticidal control, probably because it is now realized that most of them are likely to fail. The viruses are usually spread by winged aphids coming into the crop, and these insects can acquire and transmit the viruses within a few minutes. The most that can be hoped for is that the aphids will be killed before they have made so many flights as they might otherwise have done and that the failure of alatae and apterae to develop on the crop will decrease the number of plants that become infected. Emilsson & Castberg (33) sprayed potato plots with parathion at various intervals, but even weekly sprays failed to reduce the spread of potato virus Y. Broadbent, Burt & Heathcote (34) sprayed larger plots, containing Y-infected plants, fortnightly with a number of different insecticides. The virus was not introduced by incoming aphids, and spread within the crop was reduced moderately by DDT emulsion, endrin, and parathion [e.g., from 51 per cent (unsprayed) to 15 (mean of sprayed plots), 19 to 5, and 28 to 20 per cent in three different years]. The variation in percentage reduction probably reflects differences in aphid activity caused by different weather in different years. Hey (35) sprayed potatoes every four days with DDT or every two days with a parathion compound without decreasing the proportion of plants that contracted virus Y or A; sometimes spraying, by prolonging the life of plants, increased the incidence because virus was brought into the crop from nearby unsprayed potatoes which matured earlier.

Stock mosaic causes severe losses in *Matthiola incana* var. *annua*, a winter crop in California; it is brought in by *Rhopalosiphum pseudeobrassicae*

(Davis) and other aphids which acquire it from weeds. Spraying with nicotine had proved expensive and inefficient, but plots (12 ft. square) sprayed weekly with parathion contained 25 per cent mosaic, compared with 69 per cent in unsprayed plots or 56 in nicotine sprayed plots. In another year no control was obtained in single-row plots, which might reflect the inadequacy of the design but was partly attributable to a very large number of immigrant aphids [Jefferson & Eads (36)].

Most of the work on aphid control has been done to stop the spread of persistent viruses, particularly potato leaf roll. Although DDT increased yields in Maine in 1945, because the large aphid populations were reduced, it also prolonged the life of the plants and made them suitable for aphid infestation when aphids dispersed from other crops. Tubers from such sprayed crops were up to 31 per cent infected with leaf roll virus, whereas the unsprayed plots contained only 7 per cent [Bonde, Snyder & Simpson (37)]. By 1948, however, spraying or dusting of potato crops with DDT had become so general in Maine that fewer aphids were bred on infected plants, and there was less spread of leaf roll virus from field to field [Simpson & Shands (38)]. Since then most of the spread has been within the crop, and weekly applications of DDT have considerably reduced the spread of leaf roll virus [Simpson *et al.* (39)]. The combination of insecticidal treatment, early lifting, testing the health of the stock during the winter, and the planting of "foundation stock seed," has allowed the Maine seed-growers to reduce leaf roll to negligible proportions during the past few years. Another example of the value of co-operation among farmers to reduce potato virus diseases is described by Stitt & Breakey (40) and King (41). Having seen the benefits derived from insecticidal treatment on small plots during the previous year, growers in Washington started a co-operative DDT dusting programme in 1947; since then potato aphids have been few and the health of the seed stocks has improved. For example, the percentage of stocks of the variety White Rose with more than 5 per cent virus disease in 1945 was 66, but this was reduced to 3.7 in 1948 and nil in 1951, when the percentage of totally healthy stocks was 95.3 in contrast to 9.4 in 1945. Many other workers in North America found that DDT and parathion controlled aphids and increased yields and that spread of leaf roll virus from plant to plant within the crop could be reduced, but healthy crops were not produced because of aphids coming into the crops already infective [e.g., Adams & Kelley (42); Gibson, Landis & Klostermeyer (43); Fernow & Kerr (44)]. Similar results were reported by Fenjves (12) in Switzerland and Hey (35) in Germany. The amount of work done on potato aphid control is illustrated by Hill (45) who reviewed 108 papers published during the years 1944 to 1947 and stated that after a very short period of testing, DDT had largely replaced the various insecticides formerly recommended for potato insect control throughout North America.

The success that can be expected in controlling "persistent" viruses when

they are not introduced from outside, but are spread entirely from plant to plant within the crop, is illustrated by the results of Broadbent, Burt & Heathcote (34), who completely stopped leaf roll spread by spraying fortnightly with DDT emulsion, endrin, and parathion.

Parathion, TEPP, and BHC have also been successful in controlling strawberry yellows, which was the limiting factor in strawberry production in the Pacific northwest of the United States. Migrant *Capitophorus* (*Pentatrichopus*) *fragaefolii* (Cockerell) are produced mainly on mature plantings during the fruiting season, and dusting at the start of blossoming prevented these from developing. New plantings were dusted every two weeks from setting out until after harvest, and this, together with roguing, kept them healthy, whereas similar fields in other areas showed 75 per cent infected plants. The treatment of new plantings alone was insufficient, because incoming alatae were not prevented from introducing virus. The prevention of spread within the crop was confirmed by planting 10 per cent of a crop with diseased plants; dusting prevented spread from these until they were rogued 62 days later [Breakey & Campbell (46); Stitt & Breakey (40)]. As with potatoes, the growers soon adopted the control programme; since then strawberry aphids have been few and yellows scarce.

DDT emulsion failed to prevent the introduction of a virus into carrots by *Cavariella aegopodii* Scopoli, though it presumably stopped some spread within the crop, because the unsprayed plots were entirely infected, whereas the plots sprayed weekly had 68 per cent of the plants infected [Stubbs (47)]. In pea crops also, spraying with parathion did not prevent the introduction of viruses by A. *pisum* from alfalfa and clover crops, but it prevented spread within the crop of pea enation mosaic, a persistent virus, thus reducing total infection [Swenson, Davis & Schroeder (48)].

Experiments with Systemic Insecticides

One reason for contact insecticides not protecting plants against infection was the new foliage, produced between applications, which would not be fully covered with insecticide; systemic insecticides that penetrate into new growth were therefore hailed with as much enthusiasm as the introduction of DDT had been. In the first paper on the subject, Ripper, Greenslade & Lickerish (49) argued that schradan would prove superior to DDT, BHC, parathion, and other contact insecticides because its action was selective, that is, it would not harm the predators and parasites of aphids, and that these would prevent an infestation from building up again. As aphids must feed on sprayed plants before they can pick up the insecticide, it is not surprizing that systemic insecticides have been no better than contact ones in preventing either the introduction of virus into crops or the spread of "nonpersistent" viruses in crops. Almost all the work has been done on the "persistent" viruses, yellows of beet and leaf roll of potatoes. Ripper, Greenslade & Hartley (50) reported that in preliminary experiments schradan re-

duced yellows from 68 to 13 per cent, and that, even when the aphids were numerous, two or three sprays delayed the onset of the disease and thus substantially improved the yield of sugar.

Steudel & Heiling (51, 52) pointed out that control by systemic insecticides, as indeed by others, depends on the growth of the plants, the environment, and the biology of the vectors. The length of time demeton remained active varied with plant age and rate of growth and was short in young plants; incoming infective aphids infested young leaves, and these were not very toxic because the insecticide did not invade them readily. Aphid populations developed faster on open and on late-sown crops, so control was better on early-sown, closely-spaced ones. Similar numbers of beet plants became infected with yellows virus at first in sprayed and check plots, but spraying delayed further spread within the crop, e.g., in one trial the percentages of plants with yellows were as follows: unsprayed 42 on July 19, 77 on August 15 and 100 on September 10, but the comparable figures in the sprayed plots were 19, 44, and 72. Delay of virus spread was greater when virus was not very prevalent and could be delayed further by more frequent spraying. They recommended that insecticides should be applied twice or thrice with the expectation of economic advantage in areas where yellows was likely to cause serious losses but not elsewhere. A delay in onset of yellows when beets were sprayed with schradan or demeton was also reported by Ernould (53, 54). In Westfalen-Lippe demeton is applied when 30 to 50 *Myzus persicae* (Sulzer) are counted per 100 plants (in June), and a second spray is applied in July if the numbers of aphids warrant it [Dame & Goossen (55)]. In 1953 the monetary return from increased yields as a result of yellows control was about six times the spray costs.

In Britain sugar beet yellows caused seed plants to yield poorly, and the seed plants also acted as sources of virus for the root crop. Steckling beds are now either raised in specified areas where there are few sources of yellows and aphids, or they are sprayed every two to three weeks to prevent the further spread of any yellows virus that is taken into them by alatae. Control has almost doubled the seed yield, and as seedbeds with more than 1 per cent of yellows in October are not certified for transplanting, the healthy seed crops are not sources of virus during their second year when transplanted to the root-growing areas [Hull (56)]. Experiments, in which seedlings were sprayed in August, September, or October, or in combinations of two or three of these occasions, were made where a high incidence of yellows was expected. The success of the sprays depended on the time of arrival of the aphids, e.g., when aphids arrived early the early spray alone reduced yellows from 58 per cent to 36 per cent, the first two sprays (either schradan or parathion) together reduced it to 24 per cent, but the late spray had no effect. In another year the three sprays (parathion) reduced yellows from 85 per cent to 10. When aphids were numerous, however, none of the sprays adequately reduced the incidence of yellows in the stecklings, and it was concluded that spraying alone in nonisolated areas would not give sufficient

control every year [Hull & Gates (57)]. In root crops one spraying with demeton either in mid-June or early July more than halved the proportion of plants with yellows at the end of the season; spraying on both occasions had little additional effect. A third spray at the end of July decreased the proportion of infected plants by one-third. Although less than 40 per cent of the unsprayed plants became infected, spraying increased yield by from 1.8 to 3.5 cwt. per acre of sugar [Bawden (58)].

The results obtained with systemic insecticides on potatoes have varied with the district in which the experiments have been done, although it has proved impossible everywhere to prevent the introduction of either leaf roll or Y viruses [Hille Ris Lambers, Reestman & Schepers (59); Klostermeyer (60)]. Indeed, workers in areas of small-scale cultivation, such as Switzerland, where most potato crops contained many virus-infected plants, have reported increases in disease incidence after spraying with parathion or schradan, presumably because sprayed plants attract more alatae [Münster & Murbach (61); Salzmann, Schmidhauser & Meier (62)]. Rönnebeck (63, 64) also caught more alatae in traps in plots sprayed with demeton than in unsprayed plots. He found that a combination of spraying twice with demeton, roguing, and early lifting produced reasonably good seed, as most of the spread from one crop to another occurs when alate *M. persicae* leave the potatoes after the population reaches its summer maximum. He assumes that wingless aphids cause most of the spread within the crop, which is stopped by spraying, but experiments by other workers have not substantiated this idea [Hille Ris Lambers, Reestman & Schepers (59); Emilsson & Castberg (33); Broadbent (65)]. Besides using the contact insecticides already quoted, Broadbent, Burt & Heathcote (34) obtained equally good, but no better, results with fortnightly sprays of demeton, mipafox, and schradan. Another trial showed, however, that three sprays at monthly intervals with demeton controlled leaf roll as effectively as did six fortnightly sprays with parathion. Fernow & Kerr (44) also found that demeton applied every 20 days was as effective as parathion applied every 10 days.

Klostermeyer (60) immersed potato tubers in schradan and demeton and found that the plants were not colonized by aphids during their first few days above ground, but the treatment retarded plant growth, and this, with the danger of handling the tubers, precluded any practical use of the method.

Experimental Procedure

Little has been written about the design of experiments for comparing different insecticides when the control of virus spread is involved. When the vectors are transient feeders, e.g., the leafhoppers that transmit beet curly top virus, it is obvious that they will move widely over the crop, but little is known about the activity of alate insects when colonizing a crop. The only data are derived from virus disease observations, and these suggest that the alatae move short distances from plant to plant [Broadbent (65, 66)].

Whether the vectors are bringing virus into the crop or are spreading it from plants within the crop, the plots for insecticide tests need to be large or the insects will move from one plot to another (12, 16, 29, 54, 62). Hille Ris Lambers, Reestman & Schepers (59) prevented the spread of potato viruses between plots by surrounding them with two rows of oats, and Adams & Kelley (42) and Shands & Simpson in Maine separated their plots with a few feet of oats. Broadbent, Burt & Heathcote (34) adopted a different method; arguing that tests ought to be done on a crop as uniform as possible, not broken up by barriers or fallow strips which might influence insect movement, they used plots in a field uniformly planted with potatoes so that the plots were separated from each other by wide strips of unsprayed, uninfected plants. The insecticides were not likely to influence populations on other plots, but all plots would be equally liable to reinfestation by aphids from the unsprayed areas. The results of the trial would be biased against the insecticides, but if any insecticide proved satisfactory under such conditions, it could be expected to be more effective when a whole field was sprayed. The need for adequate spacing between plots was pointed out by Wilson & Sleesman (67), and Joyce (68) has recently discussed the problem of insect mobility and the design of field experiments.

The statistical design adopted may also influence the results, because the insects may bring in virus from one direction or be more numerous at one side of a field than another. For this reason randomized blocks are unsatisfactory, and a Latin Square design should be used whenever possible.

When practicable, the field should be planted with a healthy stock and an equal number of diseased plants should be introduced into each plot. With many virus diseases it is not always possible to arrange for the same proportion of plants to be infected in each plot, because, as with sugar beet yellows, the virus is always brought into the crop at random.

FUTURE PROSPECTS OF INSECTICIDAL CONTROL

In many experiments with viruses transmitted by different groups of insects there has been no evidence that applying any insecticide to a plant will prevent an infective insect from infecting it. No current insecticide that will remain effective for days on or in the foliage can kill quickly enough to prevent infection even by "persistent" viruses. But many insecticides, both contact and systemic, will kill before the insect can acquire a "persistent" virus from a plant and infect another plant within the crop, and the quicker it dies, the fewer are the plants to which it will transmit a "nonpersistent" virus. The need, therefore, is for new persistent insecticides that paralyse or kill almost immediately or for repellents that will prevent insects from feeding. Little work has been done yet on the reaction of insects to different insecticides on leaf surfaces. It is essential that not only the time taken to paralyse or kill be known but also the effect of the insecticide on insect activity, for if it irritates, but kills slowly, the insect may move and spread virus to more plants than otherwise. Infective *M. persicae* could

transmit beet yellows virus to plants recently treated with demeton or schradan, although some aphids died within half an hour [Roland (69)]. Heinze (70) found that aphids probed more frequently than usual on plants recently sprayed with demeton, and they transmitted potato virus Y more frequently to treated than to untreated plants, but this effect of spraying lasted for only a day or two. The aphids did not die after feeding for a few minutes only on the treated plants. Leaf roll virus was transmitted by 40 per cent of infective aphids to treated *Physalis floridana* plants and by 50 per cent of the aphids to untreated plants. Klostermeyer (60) found that leaf roll virus was transmitted by infective aphids to *Physalis angulata* and *P. floridana* treated with DDT, schradan, demeton, endrin, but not parathion, which killed the most rapidly. Heathcote (71) has studied the reactions of aphids on sprayed *Brassica* leaf surfaces in relation to this problem; aphids preferred leaves sprayed with a wetting agent to untreated leaves. All the insecticides tested required more than 10 min. to incapacitate an aphid, long enough for winged aphids to acquire and transmit many of the nonpersistent viruses, but not long enough to become infective with persistent viruses. None of the substances tested proved to be a satisfactory aphid repellant, and none prevented the aphid from probing the leaf.

However, good results have been, and can be, obtained with existing insecticides if the epidemiology of the disease and the ecology of the insect vectors have been studied. The effectiveness of insecticidal action can often be improved by integrating it with cultural control methods. For instance, the removal of diseased plants (roguing) to maintain the health of potato stocks was often useless because aphids arrived and spread virus before diseased plants could be diagnosed. Now that the spread of leaf roll virus can be prevented by insecticides, such plants can be removed and the health of the stock be improved. When insects invade the crop at frequent intervals over a long period, a cover of insecticide may be needed throughout the life of the plant, but often the invasions are brief and then few applications will be needed if correctly timed, and the cost will be much decreased. Now that there is more information on the mammalian toxicity and persistence of systemic insecticides in plants, they are likely to be used more widely, for some of them persist longer than contact insecticides, especially when applied to the soil. This method may be used in the future to control the spread of some tree viruses, for although there are no data yet on how they affect the spread of virus diseases, the excellent mealy bug control on cocoa obtained by applying dimefox to the soil suggests it might check the spread of the swollen shoot group of viruses [Hanna, Judenko & Heatherington (72)].

Whatever new methods are developed, however, the effective use of insecticides ultimately depends upon the attitude of the farmers. Undoubtedly many diseases of economic importance today could be reduced to negligible proportions if growers over a large area would co-operate. This applies especially where a virus is carried by insects from one crop to another;

control will always be unsatisfactory unless all the growers treat their crops to prevent insects leaving them, as has been done in various parts of the United States (38, 40, 41). When weeds are the sources of viruses government action may be needed, and the value of this has already been shown in the campaign against the beet leafhopper in the United States (23, 24). Even with farmers' co-operation and government action, many viruses diseases, perhaps most, will remain uncontrollable by insecticides until the discovery of new persistent chemicals that will kill the vectors almost instantly or prevent them from feeding.

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POLLINATION OF ALFALFA AND RED CLOVER[1]

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The subject of insect pollination is a large one with several rather distinct phases investigated by different authors. A voluminous literature has developed on floral biology with emphasis on the adaptations of flowers to particular insect visitors. Workers in this field have usually been botanists —C. R. Darwin, P. Knuth, and F. E. Clements—to name a few. The golden age for this group was in the last half of the nineteenth century. Entomologists, "behaviorists," and a few botanists have studied the instincts and adaptations of insects for utilizing floral products. Felix Plateau, August Forel, Karl von Frisch, and C. G. Butler typify this group. The greatest volume of literature in this field appeared in the first 30 years of the present century. In the last 30 years applied research directed toward improving seed and fruit production has dominated the field. Agronomists, horticulturists, and entomologists have joined forces in this effort. Insect pollination of crops is itself a broad subject with more than 50 crops involved, many of which have their own peculiar problems. This review will therefore be limited to the two most important forage crops, alfalfa and red clover.

Pollination has frequently been the principal limiting factor in the growing of alfalfa and red clover for seed. Since these are the two most important legume seed crops in the world, it is not surprising that their pollination has been studied intensively for many years. Among insects only bees serve as alfalfa pollinators to any extent. Many species of bees other than honey bees are highly efficient in this capacity, but the problem of supplying enough of them to pollinate large acreages is well nigh insurmountable in most areas. Honey bees can do a good job of alfalfa pollination under ideal conditions, but these conditions are difficult to meet and may be unattainable over certain broad regions where seed is grown. Red clover is pollinated efficiently by most kinds of bumble bees, but here again the most discouraging problem is that of supplying enough of them in the face of increasingly intensive cultivation. Fortunately, honey bees can pollinate red clover satisfactorily in most areas, providing that enough of them are used and competing bloom is not too abundant.

The research work discussed herein is concerned mainly with the manner in which the plants are pollinated, ways and means of increasing the numbers of honey bees and wild bees on the seed fields, and attempts to increase the efficiency of honey bees.

Valuable basic research on nutrition, communication, and foraging habits

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[1] The survey of the literature pertaining to this review was completed in June, 1956.

of honey bees; on the nesting habits, life histories, foraging habits of wild bees; and on nectar secretion is fundamental to all pollination research, but it would be impossible to discuss it properly in a review of this scope.

ALFALFA

TRIPPING

The peculiar mechanism of the alfalfa flower which trips, or "explodes," when a bee visits it for pollen was known at the time of Linnaeus. In 1867 Henslow (1) described the flower, the forces that hold it together, and the tensions that release the sexual column at the moment of tripping. Further descriptions of the floral mechanism were made by Müller (2) in 1873, Burkill (3) in 1894, Piper *et al.* (4) in 1914, Lesins (5) in 1950, and finally Larkin & Graumann (6) in 1954. In spite of essential agreement among these authors, there is still some question as to the exact location and nature of some of the forces involved.

Our interest as entomologists is centered more on how the bee operates the floral mechanism. This has been described in detail many times, but a simple description by Vansell & Todd (7) will suffice:

The pollen-collecting bee straddles the keel and extends its proboscis into the throat of the flower where the tripping mechanism is contacted. When the flower trips, the bee's head is momentarily caught between the standard petal and the tip of the sexual column. A splotch of pollen is entangled among the hairs on the bee's head at precisely the spot where the stigma of the next flower tripped will strike.

Before the rank and file of agronomists were finally convinced that alfalfa seed production is dependent on bees, it was necessary to prove (*a*) that tripping is required for pod setting, (*b*) that commercially acceptable varieties of alfalfa are largely cross-pollinated, and (*c*) that only bees (and rarely certain other insects) can accomplish both the tripping and cross-pollinating necessary for commercial seed production.

Müller (2) knew in 1874 that alfalfa pollen is shed in the bud stage and covers the stigma before the flower is open. His co-worker, Urban (8), found that in rare cases an untripped flower will form a pod. This was confirmed several times later, but Carlson (9) in 1930 reported as much as 26 per cent under field conditions. Kirk & White (10) in 1933, after experience with certain strains, considered that tripping was not necessary for fertilization. Brink & Cooper (11) agreed with this view in their earlier studies but later (12) retracted their statement. Most other investigators have found that with rare exceptions not more than 1 per cent of the flowers set pods without tripping [Knowles (13)]. According to Armstrong & White (14), tripping is necessary to scarify or rupture the stigmatic membrane and so release the moisture necessary for pollen germination. Petersen (15) in Denmark verified this and described the action of tripping on the membrane.

Various plant breeders [Knowles (13); Tysdal (16); Jones & Olson (17); Burkart (18)] have generally obtained figures for self-fertility ranging from

20 to 30 per cent, but extremes are as low as 5 and as high as 60 per cent. As Tysdal et al. (19) pointed out, even the self-fertile strains are largely cross-pollinated under most field conditions because of the prepotency of foreign pollen.

A number of plant breeders have tried to produce satisfactory varieties of self-fertile alfalfa [Kirk (20); Torssell (21)]. However, the selfed progeny of the strains studied have always decreased in both vigor and seed productivity. Kirk (20) found that the forage yield of Grimm fell from 100 per cent in the parent to 54 per cent in the S_4 generation. Seed yields fell to 22 per cent in the same number of selfed generations. Recently Lesins et al. (22), working in both Sweden and Alberta, have been trying to develop a moderately self-fertile variety for use in areas where insect pollination is usually insufficient. They are aiming for a plant that will trip automatically and at the same time receive enough cross-pollination from insects to retain its heterozygosity.

Automatic tripping and tripping induced by climatic agencies have been discussed by a number of authors. Interest in this subject was greatest when alfalfa was considered to be largely self-fertile [Brand & Westgate (23); Engelbert (24)]. However, Tysdal (25) in 1946 in a careful study found that rain could increase tripping but would decrease pod set and that wind had no appreciable effect. Piper et al. (4) in 1914 observed that very high temperatures could cause automatic tripping and that automatic tripping could be induced by quickly moving a raceme from a shaded location into the full sunlight. Tysdal found that flowers trip more easily on warm, bright days than on cool, damp ones. Ufer (26) in Germany concluded that neither low humidity nor high light value alone could induce tripping but that these factors aided by heat could do so. Whether continuous exposure to temperatures well above 100°F. (such as occurs in Arizona and California) could cause much tripping does not seem to have been ascertained under field conditions.[2]

Interest in automatic tripping has been revived recently in far northern areas. Lesins et al. (22) stated that automatic tripping was less desirable than insect tripping because of the smaller seed set even in moderately self-fertile varieties. They suggested automatic tripping and self-fertility only for far northern areas where conditions seem to favor automatic tripping and discourage insect pollination. Pedersen (15), Dwyer & Allman (27), and Lesins et al. (22) postulated that some cross-pollination might result from the stigma striking pollen grains already on the standard petal. They considered that grains left by nectar-collecting honey bees would be the most common but also mentioned pollen propelled by the explosive action of tripping. However, their examinations of standard petals showed that the number of such cross-pollinations must be very low.

[2] F. E. Todd in litera states that alfalfa caged without bees sets no more seed in Arizona than in Utah.

Calculations of Pollination Requirements

The populations of various pollinators necessary to set certain seed crops can be estimated from their tripping rate, their working hours, the proportion of the effective blossoming period in which they work, the number of flowers produced per unit area, the proportion of cross-pollinated flowers that set pods, the number of seeds per cross-pollinated pod, and the weight per seed. For example, Bohart *et al.* (28) calculated[3] that on a field with good agronomic potential for seed production six nectar-collecting honey bees per square yard tripping 1 per cent of the flowers visited could set about 350 pounds of seed per acre. Grandfield (29) calculated that one colony of bees tripping 2 per cent of the blossoms visited could set 120 pounds of seed. He also calculated that the nectar produced on a field is ample for at least three colonies per acre.

Although such figures are useful in showing orders of magnitude, they are subject to considerable error because of the variability of many factors involved. Flower populations vary greatly depending upon blooming conditions and the length of the effective blooming season. For example, Pedersen & Stapel (30) estimated a total production of 112,800 flowers per square meter on a field with good bloom in Denmark, and Tysdal (25) estimated 218,034 on a field in Nebraska. The proportion of cross-pollinated flowers that sets pods is nearly as variable [Hadfield & Calder (31); Tysdal (25); Petersen (15); Carlson (32)]. Pedersen *et al.* (33) showed that another cause for variability in this statistic is limitation in the ability of plants to develop pods from cross-pollinated flowers when pollination intensity is high. They found that when all the flowers on one set of plants and one-third of the flowers on another set were cross-pollinated, 46.7 per cent of the former and 66.4 per cent of the latter set pods. Menke (34) obtained similar data under field conditions. Such information shows that the effectiveness of each tripping must be lowered gradually as the total amount of tripping increases, until it finally approaches zero when the plant capacity is reached. According to Pedersen *et al.* (33), seed weight varies somewhat according to pollination intensity. They found seed averaging 2.24 mg. for a low intensity and 2.09 for a high intensity of pollination. Petersen (15) in Denmark found an average of 3.9 seeds per hand cross-pollinated pod and 1.6 per self-pollinated pod. Lesins (5) agreed fairly well with Petersen, finding 3.5 seeds per cross-pollinated pod and 1.5 per self-pollinated pod.

Another reason for caution in making theoretical calculations of the seed crop that a particular population of pollinators could set is the variation between strains of alfalfa in seed-setting potential [Petersen (15); Lesins (5)]. Finally, the calculations may be upset by indeterminate amounts of automatic tripping.

Mechanical Pollination

Seed growers have always dreamed of producing alfalfa seed without the aid of bees [Brand & Westgate (23)]. Silversides & Olson (35) in 1941

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[3] Adapted from original calculations by M. W. Pedersen.

evaluated 10 devices designed to trip the flowers and found that most of them increased tripping but that all of them decreased seed set, presumably because of injury to the plants. Several tripping machines have been built in recent years and placed on the market or used for custom operations. Pharis & Unrau (36) in 1952 tested a large machine with sponge-rubber-covered rollers working in pairs. They found that it tripped a high percentage of the flowers but failed to increase seed setting. Hvistendahl (37) recently placed on the market a "mechanical bee" designed to open the blossoms and dust the pollen ahead of the tripping device so that the stigmata will contact foreign pollen when they strike the standard petals. So far, his claims have not been substantiated by scientific investigators.

IMPORTANCE OF BEES

Urban (8) in 1873 concluded from the structure of alfalfa flowers that bees were the primary agents of pollination. At first, attention was centered on honey bees because of their abundance in alfalfa fields. However, when Henslow (1), Burkill (3), and Brand & Westgate (23) found that they tripped fewer than 1 per cent of the flowers they visited, honey bees fell into disrepute. In the United States Piper *et al.* (4) in 1914, Aicher (38) in 1917, and Sladen (39) in 1918 were among the first to emphasize that wild bees could be highly effective even when not particularly abundant. They observed species of *Megachile* tripping over 90 per cent of the flowers visited and at rates up to 25 per minute. Tysdal (16) in 1940 concluded from his studies that bumble bees were the most important pollinators in the Eastern States, leaf-cutting bees (*Megachile*) in the Great Plains area, and alkali bees (*Nomia melanderi* Cockerell) in the West. In regard to wild pollinators Tysdal's statement still holds true in a general way, but both he and his predecessors underrated the over-all importance of honey bees.

POLLINATION BY HONEY BEES

Müller (2) was the first to describe how nectar-collecting honey bees avoid the pollination mechanism of alfalfa by inserting their proboscis into the side of the flower between the standard and wing petals. Helmbold (40) observed in 1929 that some honey bees collect pollen from alfalfa and enter the flower in the normal manner.

The research in 1944–1945 by Vansell & Todd (7) and Hare & Vansell (42) in Utah laid the foundations on which the highly successful alfalfa pollination programs in California and certain other areas have been built [Townsend (43)]. Vansell & Todd (7) found that honey bee populations on alfalfa fields could be classified primarily into three groups: pollen collectors, nectar collectors operating from the side, and nectar collectors operating from the front. On the basis of later research in California, Vansell (44) described bees of the second category as "experienced" nectar collectors and of the third as "inexperienced."

Nectar collectors.—Reinhardt (45), who worked with Vansell at Davis, California, made a study of the various responses of caged honey bees to

alfalfa flowers. He classified the two types of nectar collectors as "side workers" and "nectar trippers" and showed how nectar trippers learn to be side workers and in the process lose their pollinating efficiency and nearly double their working speed. It took many of his bees several days to learn the side approach, but the scarcity of nectar trippers in the field suggests that they learn more rapidly in the open. Nectar trippers are usually observed when alfalfa first comes into bloom or when bees are first moved into the field [Pedersen & Todd (46)]. At other times they generally make up less than 1 per cent of the population.

Vansell (44) recommended "overstocking" alfalfa fields with colonies of bees to increase the percentage of young bees working close to the hives. Vansell (in personal communication) considered that it would be feasible to use recently divided colonies with rapidly expanding populations. Another method would be to move in as replacements colonies with field forces that had never worked in alfalfa.

Experienced nectar collectors accidentally trip and pollinate a small percentage of the alfalfa flowers they visit. Tremendous amounts of data accummulated by Franklin (47) in Kansas; Tysdal (16) in Ohio, Nebraska, and Wyoming; Bohart and others in Utah and California (mostly unpublished); Hobbs & Lilly (48) in Alberta; Stephen (49) in Manitoba; and many others show that this accident rate varies from time to time and from place to place. It tends to be greatest in Oklahoma, Arizona, and southern California (about 2 per cent), intermediate in Kansas, Utah, and Nebraska (about 1 per cent), somewhat lower in southern Alberta (0.7 per cent), and still lower in Manitoba (0.3 per cent). Surprisingly, this trend does not coincide with published figures for automatic tripping which have sometimes been quite high in the North [Lesins et al. (22)].

Although the tripping rates of nectar collectors are low at best, large populations tripping at the higher rates can set a fairly good seed crop. Bohart et al. (32) calculated[3] that bees tripping 1.5 per cent of the flowers they visit can set about 35 pounds of seed per acre for each bee per square yard in a three-week period. From four to six bees per square yard can be concentrated on fields with abundant bloom, and in some areas the blossoming season can be extended considerably beyond three weeks. Where nectar collectors trip only 0.3 per cent of the blossoms visited, their value must be slight, as stated by Hobbs & Lilly (48) and Stephen (49). On the other end of the scale, Jones (50) claimed that on some California fields, with yields running up to 1500 pounds of seed per acre, nectar-collecting honey bees performed 99 per cent of the tripping. In this case there must have been an extraordinarily high percentage of nectar trippers or else there were brief but unobserved periods of pollen collection by honey bees or wild bees.

Nectar collectors trip some flowers by accidentally lodging their legs in blossoms while crawling over the racemes. In such cases the stigma usually misses the bee entirely, and the result is the same as automatic tripping. This form of tripping seems to be most prevalent percentage-wise in the

North [Hobbs & Lilly (48); Stephen (49)]. Pharis & Unrau (36) found that flowers tripped by honey bees produced fewer seeds per pod than flowers crossed by hand or tripped by leaf-cutting bees and bumble bees. However, Petersen (15) in Denmark found tripping with the legs to take place in only 0.1 per cent of all visits. Furthermore, he found no difference in seeds per pod between flowers tripped by honey bees and those crossed by hand.

There is some indication that the rate of accidental tripping by nectar collectors differs between varieties of honey bees. Petersen (15) reports that in Denmark "*Apis mellifica*" (the native, dark German or Nordic bee) trips about 2.8 per cent of the flowers visited and "*Apis ligustica*" (the Italian variety) trips about 2.1 per cent. These are surprisingly high tripping rates, but they may include some activity by nectar trippers. Bieberdorf (51) in Oklahoma found indication that Caucasians were more effective trippers than Italians. Apparently, no one has done any work on this problem from the standpoint of bee breeding.

McMahon (52) in Saskatchewan made the unique observation that accidental tripping by nectar collectors increased from 0.3 per cent to 1.8 per cent when the removal of surrounding bloom greatly increased the bee population on the field. He found that when the field was "overstocked" the bees had to work the compactly clustered florets toward the apex of the raceme and in doing so were forced to enter many flowers from near the front, thus increasing the accident rate. McMahon suggested two methods of achieving such high populations: increasing the number of colonies per number of flowers and eliminating competing bloom. He failed to point out, however, that exceptionally high populations will not visit a field unless nectar production is also exceptionally high [Pedersen (53)].

Pedersen & McAllister (28) found that open stands had twice as much nectar per flower, twice as many bees per flower, and twice as much seed per acre as dense stands. It may well be that the principal value of open stands lies in the heavy concentrations of honey bees attracted by the copious secretion of nectar in well spaced plants.

Other methods of increasing the number of bees on the field have been suggested. Drake (54), on the basis of studies in Iowa and Nebraska, strongly recommended growing seed from part of the field as the first growth and staggering the cutting dates on the rest of the field to cause the appearance of second-crop bloom at different times over the field. Todd (55) suggested the same method of utilizing the bee supply more effectively. In some areas staggering the cutting dates would increase weed and insect control problems. Soboleva (56) in Russia advocated feeding bees 50 to 100 gm. of alfalfa-scented sugar syrup per hive for 5 to 10 days at the time of mass flowering.

Vansell (44) and Jones (50) reported that when colonies of bees were first placed in central California alfalfa fields the population rose, but after about eight days it fell to near the original level. When these colonies were replaced by others, the same thing occurred. Levin (57, 58) observed that

bees from newly placed colonies visited alfalfa more freely than those from colonies already in the field, whether or not they had had previous experience with alfalfa. In general, the newcomers stayed with the alfalfa longer when moved from an area with abundant alfalfa to one with little alfalfa.

Akerberg & Lesins (59) postulated another role for nectar collectors, that of "setting up" the flowers for automatic tripping by their repeated visits. They found that when bee trippings were subtracted from total trippings in their unguarded plots the remainder was still greater than the amount of tripping in guarded plots. Pharis & Unrau (36) corroborated Lesins' findings in cages with and without honey bees. However, Piper et al. (4) concluded from experiments at Pullman, Washington, that nontripping insects had no effect on subsequent pod set. If such delayed-action tripping is prevalent, it might explain the difficulties that we in northern Utah have always had in trying to associate nectar-collecting honey bee populations with the number of tripped flowers from hour to hour. The Swedish investigators (59) believe that it may also explain the rather large quantity of selfed seed they harvest from fields where wild bees are scarce.

Pollen collectors.—Pollen-collecting honey bees are far more desirable on alfalfa seed fields than those in either of the nectar-collecting categories. Bohart et al. (28) pointed out that, since pollen collectors trip about 80 per cent of the blossoms they visit compared with 1 per cent for nectar collectors and visit slightly over half as many flowers per minute, they should be 45 times as valuable. However, since they "take time off" now and then to gather nectar for their own needs, their relative efficiency is probably somewhat less than this. Nevertheless a few pollen collectors added to a nectar-bee population can make a big difference in the rate of pollination. The percentage of pollen-collecting honey bees found on different seed fields varies from 0 to 100. It may vary from 1 per cent to 50 per cent on adjacent fields depending upon the growing condition of the plants (unpublished observations). Table I shows the percentages of pollen collectors on alfalfa in various localities. These figures follow the same regional trends as those for tripping by nectar collectors. The reasons for this are not clear, but they probably involve a combination of climatic effect on the plants and the nature and abundance of competing pollen sources [Bohart (61)].

Many investigators have stressed the importance of competing pollen sources [Linsley & MacSwain (62); Vansell & Todd (7); Hare & Vansell (42); Franklin (47); Hobbs & Lilly (48); and others]. That honey bees can be forced to collect alfalfa pollen by caging them inside plots of alfalfa has been demonstrated by several workers [Akerberg & Lesins (59) in Sweden; Hobbs & Lilly (48) in Alberta; Reinhardt (45) in California; Levin & Pedersen (64) in Utah]. Linsley & MacSwain (62) increased the number of pollen collectors on an alfalfa field by cutting an adjacent field of mustard but only after the entire field was cut. Similar trials in Utah failed [Bohart (61)]. In this case the competition was in the form of continuous strips of roadside gumweed and sweetclover, and the bees merely moved along the same strips to points beyond the mile zone of control.

There is some evidence that certain pollen sources for honey bees such as sweetclover and mustard, are more potent as competitors than others such as greasewood (*Sarcobatus*) and various grasses [Hobbs & Lilly (48); Hare & Vansell (42)]. The relative potency of the competing sources may be as important as their abundance in determining the degree to which they need to be controlled [Bohart (61)].

Climate and competition are not the only factors controlling abundance of pollen collectors on alfalfa. Hare & Vansell (42) were the first to note the importance of plant condition in attracting pollen collectors. Vansell (44) suggested that lush conditions on certain fields at Delta, Utah were responsible for low amounts of tripping. Unpublished observations by the Legume

TABLE I

PERCENTAGE OF POLLEN-COLLECTING HONEY BEES IN VARIOUS LOCALITIES*

Locality	Observers	Per cent of Pollen Collectors
Southern California	Linsley & MacSwain (62)	5–20
Southern Arizona	Bohart	4–100
North cental California	Linsley & MacSwain (62)	0–5
Central Utah	Vansell & Todd (7)	5–50
Northern Utah:		
Howell Valley	Bohart & Levin	1–50
Cache Valley	Bohart & Pedersen	
	Vansell & Todd (7)	0–5
Wyoming	Bohart	0–1
Washington	Menke (34)	0
Alberta	Hobbs & Lilly (48)	0
Manitoba	Stephen (49)	0
Minnesota	Haws (63)	0
Saskatchewan	Peck & Bolton (60)	0

* Figures given for Bohart and associates are taken from unpublished data.

Seed Research Laboratory at Logan, Utah, generally support the same conclusion. Pedersen & Bohart (65), in a study of the attractiveness of certain clones of alfalfa to pollen-collecting bumble bees, found nectar production to be the most highly associated. It might be expected, therefore, that high nectar production would be associated with the slightly dry condition that pollen-collecting honey bees seem to favor. However, other factors are probably involved because, according to Pedersen (unpublished data), only when the soil moisture is exceptionally high does nectar production decline.

Rudnev (41) claimed that he increased pollen collection by honey bees in Russia both by replacing pollen-filled combs with empty combs and by feeding syrup flavored with alfalfa pollen. The reported increases appear to have been for all pollen rather than alfalfa pollen alone. Butler & Simpson

(66) failed to increase the collection of red clover pollen in the field by suspending red clover pollen in syrup fed to the bees. Consequently, they doubted that "training" would serve to increase pollen collection.

Recommendations.—Todd & Vansell (67) gave some practical advice for alfalfa seed growers interested in using honey bees for pollination. They recommended three colonies or less per acre for fields where pollen-collecting honey bees were abundant and six colonies or more for fields depending on nectar trippers.[4] They also recommended scattering the colonies in groups of 10 to 12 throughout the fields. To take full advantage of the higher tripping occurring around the incoming colonies, they suggested moving in the first colony per acre at the $\frac{1}{4}$ bloom stage and the remainder at intervals of 7 to 10 days until five to six bees per square yard are present at the peak of bloom. Considering the tremendous variation in the working habits of honey bees on alfalfa, it is probably impossible to make satisfactory blanket recommendations for colony numbers. Bohart *et al.* (28) in 1955 recommended a formula similar to that of Todd & Vansell and further suggested methods by which the grower could check his progress and adjust the number of colonies.

Where there is no pollen collection and tripping rates range around 0.5 per cent, it seems to be doubtful whether honey bees can be utilized on an economically sound basis [Stephen (49)]. Pengelly (68) suggested that in such areas honey bees should not be used because they lower the attractiveness of the field and seriously compete with wild bees in poor forage years and during a critical season. He quoted an ecological paper by Pearson (69) to support his belief. Peck & Bolton (60) expressed a similar view. Bohart *et al.* (28) stated that heavy concentrations of honey bees lower the attractiveness of alfalfa fields to bumble bees and leaf-cutting bees but not to alkali bees. They recommended that if the field normally attracts enough wild bees (except alkali bees) to set a good seed crop, honey bees should not be increased in the area. On the other hand Jones (50) stated that honey bees do not depress populations of other bees.

The evidence in favor of Todd & Vansell's recommendation for scattering colonies throughout the fields is not yet conclusive. Vansell (44) reported that a field in California set 30 per cent more seed 100 feet from the colonies than 1,000 feet farther out. He attributed the phenomenon to greater numbers of young, inexperienced bees close to the colonies (personal communication). This belief is based on the assumption that young bees and bees recently moved into a field tend to work close to the hive. Members of the research group at Logan, Utah, are now attempting to verify this point.

POLLINATION BY BEES OTHER THAN HONEY BEES

Judging from the 50 or more species of alfalfa-visiting bees found in Utah [Bohart (28)], the total number on alfalfa the world over must be

[4] Experienced nectar collectors were not mentioned but may have been omitted by oversight.

several hundred. The problem of reviewing the scattered literature on the pollinating activities, populations, seasonal and geographic distributions, and methods of protecting, increasing, and utilizing even the most important members of this assemblage leaves me with a feeling of helplessness to say the least.

Linsley (70) in California wrote the first survey paper for the wild pollinators of any area. Subsequently Drake (54) in Iowa, Peck & Bolton (60) in Saskatchewan, Stephen (49) in Manitoba, Pengelly (68) in Ontario, Franklin (47) in Kansas, Fischer (71) in northern Minnesota, Bohart (72) in Utah, Menke (34) in Washington, and Hobbs & Lilly (73) in southern Alberta have made at least hasty surveys of local areas. As might be expected, the largest number of species are found in the bee-rich areas west of the Rockies. Lesins (5) in Sweden, Petersen (15) in Denmark, and Ufer (74) in Germany have published considerable information on the limited number of species of wild bees pollinating alfalfa in northern Europe. So far as I know, the wild bees visiting alfalfa in southeastern Europe and central Asia, where alfalfa supposedly originated, have not been surveyed.[5]

Table II summarizes existing information concerning the species known to have at least local or sporadic importance. Obviously there are many unreported species of importance in seed-growing areas such as Argentina, southern Russia, and Australia. I have taken the liberty of "interpreting" some of the published data to make it conform to the headings used in the Table. For example, my observations indicate that bumble bees and *Megachile* nearly always trip alfalfa flowers when they are seeking pollen. Consequently, when only 30 per cent of the visits of a species of these genera were reported as causing the flower to trip, the table shows that about 65 per cent were collecting nectar.

It can be seen at once that generalizations concerning pollination by wild

[5] Since writing the above, two important Russian articles by V. B. Popov have come to my attention. The first ["The Significance of Bees (Hymenoptera-Apoidae) in the Pollination of Alfalfa," *All-Union Entomol. Soc.*, **43**, 65–82 (1952] lists 22 important alfalfa pollinators in Central Asia as follows:

Halictus eurygnathus Bluthgen, *H. malachurus* (Kirby), *Andrena labialis* (Kirby), *A. flavipes* Panzer, *A. albofasciata* Thomson, *Nomia diversipes* Latreille, *Melitta leporina* (Panzer), *Melitturga clavicornis* (Latreille), *Megachile argentata* (Fabricius), *M. pilidens* Alfken, *M. maritima* (Kirby), *M. saussurei* Radoszkowski, *Eucera tuberculata* (Fabricius), *E. interrupta* Bär, *E. clypeata* Erichson, *E. nigrifascies* Lepeletier, *E. chrysopyga* Pérez, *Tetralonia tricincta* Eversmann, *Amegilla magnilabris* (Fedtschenko), *Bombus silvarum* (Linnaeus), *B. agrorum* (Fabricius), *B. terrestris* (Linnaeus).

The second ["Bees, Their Relations to Melittophilous Plants and the Problem of Alfalfa Pollination," *Entomol. Revs. U.S.S.R.*, **35**, 528–98 (1956)] summarizes knowledge on the distribution and biology of the most important species of central Europe and Asia. *Melitturga clavicornis* (Latreille) was considered the most valuable species. Honey bees were given little credit for alfalfa pollination in any part of Russia.

bees are rather futile because of the great diversity among them. Even within a genus and in some cases a species, it is difficult to make valid generalizations. For example, *Megachile frigida* Smith is considered by Stephen (49) and Peck & Bolton (60) to be the most important alfalfa pollinator in Manitoba and Saskatchewan. Stephen claims that it prefers alfalfa to all other host plants. On the other hand, Hobbs & Lilly (73) say this species is rather common in southern Alberta but is almost never found on alfalfa. In Utah I have seen it commonly on hairy vetch and wild licorice (*Glycorrhiza*) but never on alfalfa. Stephen found *Megachile brevis* Say to be principally a nectar collector on alfalfa in Manitoba. Linsley (70) found it to be a consistent and efficient collector of alfalfa pollen in California.

In spite of these precautions, the following generalizations, compounded from the literature and personal experience, may be in order: (*a*) Wild bees more than $\frac{3}{8}$ inch long are generally much more consistent trippers of alfalfa than are honey bees. Bees less than $\frac{1}{4}$ inch long do not trip at all [Bohart *et al.* (28)]. (*b*) With the exception of *Nomia melanderi* Cockerell, wild bees rarely keep pace with expanding acreage of seed. Sooner or later the combined effects of reducing the nesting areas and spreading the existing population over more alfalfa reduces their effectiveness. (*c*) With the exceptions of *Nomia melanderi* in suitable habitats and certain species of *Megachile* in the cottonwood and aspen "bush country" of Canada, we have no proven methods for large-scale increase of wild alfalfa pollinators [Todd & Vansell (67)]. (*d*) Because wild bees often have short seasons and sporadic or cyclical appearance from year to year, it is advantageous in each area to have as many alfalfa-visiting species as possible. Consequently, there should be great possibilities for improving the pattern of wild bee pollination by making suitable introductions [Piper *et al.* (4); Larkin (76)].

Among exceptions to point (*a*) are certain long-tongued bumble bees and *Anthophora* that visit alfalfa largely for nectar and avoid the pollinating mechanism in the same manner as nectar-collecting honey bees [see Ufer (74) concerning *Bombus* and Stephen (49) concerning *Anthophora*]. Bees of this sort can sometimes avoid tripping the flowers even when they enter the throat of the flower. Small halictids and andrenids often have great difficulty tripping the flowers and spend much of their time looking for tripped flowers [Franklin (47)]. However, when they do succeed in tripping, they do it more frequently than nectar-collecting honey bees. In general, large size is correlated with speed in tripping. Queen bumble bees work more rapidly than workers [Franklin (47)], and large individuals of *Halictus rubicundus* Christ trip more flowers than small ones (unpublished data).

Linsley (70) suggested that the species of *Lasioglossum, Halictus*, and *Hylaeus* that are too small to trip flowers may be of benefit in cross-pollinating automatically tripped flowers. Pengelly (68) discussed the same possibility. However, Pedersen (verbal communication) concluded that cross pollination after tripping probably occurs but rarely, since the entire receptive surface of the stigma is tightly appressed to the standard petal. If Linsley's hypothesis is valid, tripping machines might be used to advantage in areas

TABLE II

Wild Pollinators of Alfalfa*

Species	Areas Important as Alfalfa Pollinators	Alfalfa a Preferred Host	Per Cent Collecting Nectar	Flowers Tripped by Pollen Colectors		Nesting Sites
				Per Cent	Number Per Minute	
Bombus terrestris (Linn.)	Northern Europe (L), (Pe), etc., New Zealand (Ha)	Yes (Le)	10–30 (Pe)	70–90 (Pe)	20 (Le)	Underground, usually deep (Lo)
occidentalis Greene	Great Basin, U.S. (B), Southern Alberta (H)	Yes (B)	O (B)	90–95 (B)	15–18 (B)	Underground, in rodent nests (B)
terricola Kirby	Manitoba (S), Minn. (Fi), Saskatchewan (P), Ontario (Pn)	Yes (S)	30–40 (Pn)	85–90 (S)	12–28 (P)	Underground long tunnel (Pl)
distinguendus Morawitz	Central Denmark (Sk)	No (Sk)		86 (Sk)		Underground (Lo)
borealis Kirby	Saskatchewan (P)		60± (Pn)		12–27 (P) 19+(Pn)	Underground (Pl)
morrisoni Cresson	Great Basin of U.S. and Southern Utah	Yes (B)	5–10 (B)	80–95 (B)	16–22 (B)	Above ground in buildings, etc. (B)
crotchii Cresson	California (L)					
huntii Greene	Great Basin of U.S. (B)	No (B)	20–40 (B)	80–90 (B)	14–18 (B)	Underground in rodent nests (B)
griseocollis DeGeer	Utah and Idaho (B)	Yes (B)	10–20 (B) 40–50 (Pn)	90± B	15–20 (B)	Above ground or at surface (B)
ternarius Say	Manitoba (S), Ontario (Pn)		55± (S) 40–50 (Pn)		20–24 (Pn)	Underground long tunnel (Pl)
impatiens Cresson	Iowa (D), Ontario (Pn)	No (Pn)	20–25 (Pn)		16–22 (Pn)	Underground long tunnel (Pl)
vagans F. Smith	Manitoba (S), Saskatchewan (P)	No (P)	80± (S)		15–16 (S)	Some surface, some underground (Pl)
californicus F. Smith	California (L)					
sonorus Say	California (L)					
americanorum (Fabricius)	Kansas (F), Ontario, (Pn)		50± (F)	80 (Pn)	16–20 (Pn)	Above ground, shallow underground (Pl)
erridus (Fabricius)	Manitoba (S), Ontario (Pn)	No (B)	90± (B) 50± (B)	80–90 (B)	21 (B) 14–28 (Pn)	Near surface of ground —above or below (B)
fraternus (F. Smith)	Kansas (F)		50± (F)			
rufocinctus Cresson	Manitoba (S), Saskatchewan (P)	No (P)	60± (S)		12–27 (P)	Above ground or at surface (Pn)
auricomus (Robertson)	Iowa (D)					Shallow underground (Pl)
Anthophora furcata terminalis Cresson	Saskatchewan (P), Ontario (Pn)	No (Pn)	60± (Pn)		12 (B) 14–17 (Pn)	Decayed wood (B)
urbana Cresson	California (L), Southern Utah (B)	No (B)	40±	86 (B)	10–14 (B)	Sandy soil — usually banks. Gregarious (B)
californica Cresson	Southern Calif. (L)	No (L)				Soil (B)
walshii Cresson	Iowa (D)					Soil (B)
Tetralonia edwardsii Cresson	Utah, Idaho (B)	Yes (B)	0 (B)	97 (B)	14–16 (B)	Soil-hidden in grass (B)
Eucera longicornis (Linnaeus)	Northern Europe (Le), (Pe), etc.		O (Le)		7–15 (Le)	Soil (Le)
Melissodes agilis Cresson	California, Kansas (L), (F)	No (L)	40 (B)	60–85 (B)		Soil (B)
timberlakei Cockerell	California (L)		30 (B)		16–19 (B)	Soil—under chips or other objects (B)
obliqua (Say)	California (L), Kansas (F)	No (B)				Soil—in sparse grass (B)
Florilegus condignus (Cresson)	Nebraska (La), Iowa (D), Kansas (F)	No (La)	60± (D)	95 (LA)	20 (La)	Soil (B)
Xylocopa virginica (Linnaeus)	Kansas (F), Iowa (D)					Dead wood (B)

TABLE II—(*continued*)

Species	Areas Important as Alfalfa Pollinators	Alfalfa a Preferred Host	Per Cent Collecting Nectar	Flowers Tripped by Pollen Collectors		Nesting Sites
				Per Cent	Number Per Minute	
varipuncta Patton	California (L)					Dead wood (L)
californica arizonensis Cresson	Southern Utah (B)	Yes (B)	0 (B)	90± (B)	23–30 (B)	Dead wood and yucca stalks (B)
sp.	Israel (me)					
Megachile brevis (Say)	Calif. (L), Utah (B), Alberta (H), Manitoba (S), Ontario (Pn)	Yes (B) No (S)		99–100 (Pn)	16–20 (Pn)	Hollow and pithy stems (B)
onobrychidis Cockerell	Utah (B), California (L)	Yes	0 (B)	98 (B)	18–21 (B)	Soil cracks under clods, stones (B)
gentilis Cresson	Utah (B), California (L)					Soil (B)
coquilletti Cockerell	Utah (B), California (L)			98± (B)		Probably soil (B)
mendica Cresson	Iowa (D)					
texana Cresson	Utah (B), Ontario (Pn), Kansas (F)	Yes (B)	0	95 (B) 85–95 (F)	16–20 (B) 18–22 (Pn)	In soil (Pn)
perihirta Cockerell	Western U.S., Western Canada	Yes (B)	0 (B)	98 (B)	19–24 (B)	In soil, between hay bales, in gravel (H), (B)
dentitarsus Sladen	Utah, Idaho, Alberta	Yes (B)	0 (B)	95± (B)	18–26 (H)	In soil with light vegetation (H), (B)
latimanus Say	Minn. (Fi), Man. (S), Saskatchewan (P), Ontario (Pn)	Yes (P)	0 (P)	95 (P)	16–22 (P) 12.2 (Fi) 19–28 (Pn)	In soil (P)
relativa Cresson	Minn. (F), Man. (S), Saskatchewan (P)	No (P)	40 (S)		"rapid" (P)	Tunnel in bank, possibly in wood also (P)
nivalis Friese	Saskatchewan (P)	No (P)			"rapid" (P)	Beetle holes in cottonwood, etc. (P)
inermis Provancher	Manitoba (S), Saskatchewan (P)	No (Pn)	0 (Pn) 60 (S)	95 (Pn)	16–22 (Pn) 8–27 (P)	Old cottonwoods (P)
melanophaea Smith	Manitoba (S), Saskatchewan (P), Ontario (Pn)	No (S)	40 (S) 0 (Pn)	100 (Pn)	13–16 (Pn) 15 (P)	
gemula Cresson	Minn. (Fi), Saskatchewan (P)	No (P)			11.4 (Fi)	
integra Cresson	Kansas (F)		5 (F)	85 (F)		
willugbiella (Kirby)	Northern Europe, (Le), (Pe)		0 (Le)	100 (Le)	20 (Le)	Gravel, holes in soil (Le)
Diceratosmia subfasciata punctata Michener	Imperial Co. Calif. (L)	Yes (L)		High (L)		Beetle burrows in wood-willow thickets, etc. (L)
Osmia seclusa Sandhouse	Utah, Idaho (B)	Yes (B)	0 (B)	95 (B)	12–14 (B)	Soil in nest of *Diadasia* (B)
Anthidium edwardsii Cresson	California (L)	No (B)	0 (B)	95 (B)	14–18 (B)	Shallow burrows in soil (L)
Halictus farinosus Smith	California (L), Utah (B)	No (B)	0 (B)	60–90 (B)	6–9 (B)	Hard-packed soil. Cells 4–15 in. deep (B)
Halictus rubicundus Christ	Utah (B)	No (B)	0 (B)	10–60 (B)	1–5 (B)	Hard or medium soil. Cells 6–12 in. (B)
ligatus Say	California (L)	No (B)	0 (B)			Hard-packed soil (L)
parallelus Say	Iowa (D)	No (D)	0 (B)			Hard-packed soil (B)
Lasioglossum athabascense Sandhouse	Ontario (Pn)		0 (Pn)	"low" (Pn)	"low" (Pn)	
sisymbrii Cockerell	Utah (B)	No (B)	0 (B)	30–60 (B)	3–8 (B)	Soil (B)
Rophites canus Eversmith	Central Europe (U), (Le)	Yes (U)	0 (U)	90–95 (U)		Soil (U)

TABLE II—(*continued*)

Species	Areas Important as Alfalfa Pollinators	Alfalfa a Preferred Host	Per Cent Collecting Nectar	Flowers Tripped by Pollen Collectors		Nesting Sites
				Per Cent	Number Per Minute	
Nomia melanderi Cockerell	Western U.S. (B) etc.	Yes (B)	0 (B) Except Males	90–95 (B) (males 40–70)	10–14 (B)	Moist, alkaline soil. Cells 6–9 in. (B)
Nomia nevadensis Cresson	Southern Calif. (L)	No (B)	0 (B)	90± (B)	10 (B)	Moist, usually alkaline soil. Cells 6–20 in. deep (L)
Agapostemon cockerelli Crawford	Utah (B), California (L)	No (B)	0 (B)	40–80 (B)	6–10 (B) Males 1–5	Deep in hard soil (L)
melliventris Cresson	Southern Calif. (L)		0 (B)			Soil (L)
virescens (Fabricius)	Iowa (D), Utah (B)	No (B)	0 (B)	50–80 (B)	8–10 (B) Males 1–5	Deep in soil, sometimes in lawns (B)
Andrena wilkella (Kirby)	Indiana, Ontario (Pn)		0 (B)	40–60 (Pn)	11–18 (Pn)	Soil (B)
prunorum Cockerell	Utah, Idaho (B)	No (B)	0 (B)	40–60 (B)	4–8 (B)	Loose soil (B)
Nomadopsis scutellaris (Fowler)	Utah (B)	No (B)	0 (B)	5–40 (B)	1–5 (B)	Hard-packed soil, often moist (B)
Calliopsis andreniformis (Smith)	Nebraska (La, Cr)		0 (Cr)			Hard-packed soil (Cr)
Melitta leporina (Panzer)	Northern Europe	Yes	O (Le)	90–100 (Le)	15 (Le)	Soil, entrances concealed (Le)
Macropis labiata (Fabricius)	Central Europe (U)					Soil (Lo)
Campsomeris plumipes (Drury)†	Southern Utah (B)	No (B)	eat pollen nectar (B)	52 (B)	4–9 (B)	Parasite of scarab larvae in soil (B)
tolteca (Saussure)†	California (L), (Hu)					
Chauliognathus pennsylvanicus DeGeer‡	Iowa (D)	No (D)				Predator on aphis, etc (B)

* L=Linsley (70), Pe=Petersen (15), Ha=Hadfield & Calder (31), Sk=Skovgaard (75), B=Bohart (mostly original), F=Franklin (47), S=Stephen (49), Fi=Fischer (71), P=Peck & Bolton (60), D=Drake (54), Le=Lesins (5), La=Larkin (76), Me=Melamed (*in lit.*), U=Ufer (74), Cr=Crandall & Tate (77), Pn=Pengelly (68), Pl=Plath (78), Lo=Loeken (*in lit.*), Hu=Hurd (140).
† Scoliidae
‡ Cantharidae

where these bees are particularly abundant. In Utah it has been observed that *Halictus provancheri arapahonum* Cockerell often increases on a field as soon as large-scale tripping takes place.

Stephen (49) described the usual history of seed production in new areas. At first there are a few small seed fields with exceptionally high yields; a period of land expansion, clearing of burnt-over land, and thorough cultivation follows; finally, within 4 to 10 years yields drop from 1,000 pounds to 150 pounds or so, and seed production is no longer profitable. According to personal communication from Wayne Wright, a large-scale seed grower in northern Manitoba, the usual history has been interrupted and perhaps even reversed in this territory by a process of limiting fields to narrow strips surrounded by aspen and cottonwood "bush." According to reports, the growers are providing nesting sites for *Megachile* and bumble bees by piling refuse

timber around the edges of the fields. Obviously such a procedure is feasible only under special conditions.

The usual history has likewise been altered in certain seed-growing districts of the Northwest where conditions are favorable for *Nomia melanderi*. The early expansion of seed production in these areas, instead of reducing *Nomia* populations, has actually increased them by providing large amounts of sweetclover, alfalfa, and other plants to replace the former meager forage [Bohart (79)]. Furthermore, irrigation has greatly increased areas of alkaline, waterlogged land suitable for their highly gregarious nesting. In such areas many seed growers are protecting their nesting sites and certain farmers are developing new nesting sites [Menke (34, 80); Bohart (79, 81, 82); Bohart & Cross (83)].

General rules for making the best use of wild bees were summarized by Bohart *et al.* (28) as follows: (*a*) Time the bloom with the period of their greatest abundance. (*b*) Plant seed alfalfa in areas where alfalfa-pollinating species are known to be abundant [McMahon (84)]. (*c*) If wild bees are setting most of the crop, don't expand the acreage beyond the capacity of the bees to pollinate it [Stephen (49); Bohart (85)]. (*d*) Reduce competing bloom in the area during the period the seed crop is in bloom. (*e*) Provide spring and early-summer bloom for bumble bees, leaf-cutting bees, and other species with long seasons [Pengelly (68); Bohart & Knowlton (86)]. (*f*) In areas with natural timber growth, provide nesting sites for leaf-cutting bees by cutting and piling trees (especially poplars, willows, and aspens) near the alfalfa field. The timber should be piled loosely for aeration and availability to beetles and bees. (*g*) Search for nesting sites of gregarious species and keep them in an unaltered condition.

HIGHLIGHTS

The highlights of alfalfa pollination may be summarized as follows: (*a*) Existing commercial varieties depend upon bees to trip and cross-pollinate enough flowers to produce a commercial seed crop. However, some efforts are still being made to develop satisfactory self-tripping and self-fertile varieties. (*b*) Honey bees are efficient alfalfa pollinators when they collect pollen but inefficient when they collect nectar (except during a brief learning period). (*c*) In areas where the weather is cool or humid or both, or where strongly competing pollen sources are abundant, honey bees rarely collect alfalfa pollen. (*d*) Pollen collecting can be encouraged in suitable areas by spacing the plants and not irrigating heavily after the bloom begins. (*e*) Nectar-collecting bees can set a good seed crop if sufficiently abundant on the field. If they trip many fewer than 1 per cent of the flowers, the population sufficient for setting a good seed crop may be unattainable. (*f*) Most species of wild bees are highly efficient pollinators when they visit alfalfa, but in most areas they are too scarce to set good seed crops. Practical methods for conserving and increasing them are still undeveloped except in the cases of *Nomia melanderi* in the northwest and timber-inhabiting *Megachile* in the cottonwood and aspen zones of Canada.

RED CLOVER

Tripping

The floral structures of red clover and alfalfa differ significantly with respect to their tripping mechanism. In alfalfa, if cross-pollination doesn't take place during the single act of tripping, it is not likely to take place at all. The pollinating mechanism of red clover is of the piston type. Pressure against the standard and wing petals operates a lever which forces the stigma and anthers upward and out of the enclosed keel petals. When the pressure is released the sexual parts revert to their former position. There is no naturally occurring side opening that bees can use for stealing nectar without tripping the flowers. Therefore, any insect that applies sufficient pressure to the petals when seeking either nectar or pollen serves as a pollinator. With few exceptions, only bees apply such pressure. Müller (87) described the floral mechanism in considerable detail.

Darwin (88) popularized red clover pollination with his assertion that cats increase red clover seed yields by catching mice that destroy nests of the bumble bees that pollinate red clover. Hardly an article on red clover seed production neglects to mention Darwin's concept, although it is based on two controversial assumptions, (a) that red clover depends upon bumble bees for pollination and (b) that mice reduce bumble bee populations.

Darwin's insistence that red clover requires insect pollination was disputed by Meehan (89) but was later dramatically proven, by the introduction of bumble bees into New Zealand and the subsequent abrupt increase in seed production. Since honey bees were already present this event also seemed to confirm Darwin's belief that honey bees are of little value to red clover.

Until the intensive studies of Westgate & Coe (90) in 1915, opinion was about equally divided as to whether red clover is self-sterile or self-fertile. The need for insect pollination was well recognized, and the predominance of bumble bees as pollinators was accepted by all except a few workers such as Hopkins (91) in 1896 and Pammel & King (92) in 1911. Westgate & Coe showed that all commercial varieties of red clover were self-sterile, but they failed to convert many workers to the view that honey bees are important as pollinators. As late as 1925 Plath (93) reported seeing no honey bees in a four-year study of a red clover field near Boston.

Pollination by Honey Bees

More recent workers have been generally in agreement that honey bees are efficient red clover pollinators under the proper conditions. The first reason advanced for the reluctance of honey bees to visit red clover was the long, narrow corolla tube of the plant in comparison to the length of the honey bee's proboscis [Pammel & King (92)]. Martin (94) in 1938 calculated that with corolla tubes 8.5 mm. in length honey bees could just touch a nectar column 1 mm. high. This, he said, placed most American varieties out of the reach of honey bees for nectar collection. However, Pedersen (95)

found that the height of the nectar column varies from 0 to 1.5 mm. in bee-visited flowers and from 0 to 3.5 mm. in isolated flowers. Wexelsen (96) pointed out that the value of honey bees was reduced by their habit of seeking out old florets, incapable of fertilization but wilted enough for the bees to reach the nectar. Another drawback of honey bees is their habit of "stealing" nectar from the holes cut in corolla bases by certain species of bumble bees [Pedersen (95); Schelhorn (97)].

Investigators have perceived three obvious approaches to the problem of fitting honey bees to red clover flowers: (a) shorter corolla tubes, (b) more nectar, (c) longer-tongued bees. Lindhard (98) and Kratochvil & Snoflak (99) obtained greater yields from short tubed red clovers than from long-tubed varities. On the other hand, Wilsie & Gilbert (100) found the latter more attractive to honey bees and higher in seed production. This discrepancy may have been associated with holes that short-tongued species of bumble bees in Europe had cut in the corollas of long-tubed strains. Starling *et al.* (101) on the basis of earlier studies and a genetic study concluded that the short-tubed strains had no advantage over the long-tubed ones in seed setting and that they were lower in general growth and vigor. In spite of these results Åkerberg *et al.* (102) consider the development of a good short-tubed variety a major objective.

Cultural rather than genetic attainment of a plant with short corolla tubes has been more widely accepted. As early as 1906 Fruwirth (103) quoted Schachinger as saying that corolla tubes are shorter in the second crop than in the first. Akerberg (104) found a direct correlation between hot, dry weather and short corolla tubes. Pedersen (95) in Denmark, Åkerberg *et al.* (102) in Sweden and Dunham (105) in Ohio reported a greater visitation of honey bees to the short-tubed flowers of second crop. Pedersen's data indicate three other possible reasons: (a) There was slightly more nectar per floret in late clover. (b) There were more flower heads per unit area and more florets per head on late clover. (c) There were only half as many bumble bees on late clover. This reduced the competition, thus allowing for an increase in honey bees. It also reduced the number of holes cut in the corollas by bumble bees. In spite of such evidence in favor of growing second crop for seed, Wilsie & Gilbert (100) and Starling *et al.* (101) found no increase in seed setting of the second crop over the first. Hammer in 1949 (106) and Shuel in 1951 (107) found that red clover secretes nectar most copiously in hot weather and that the sugar concentration is highest in hot, dry weather (through increased evaporation). Since the atmosphere is generally hottest and driest in late July, more favorable nectar supply rather than shorter corollas may be the reason for the attractiveness of second-crop bloom to honey bees.

According to Stapel (108), Italian bees are more consistent visitors to red clover than northern European races because their tongues are slightly longer. Schwan (109) in Sweden found the short-tongued Nordic bees more disposed than Italian bees to use the holes cut by bumble bees. However, he

found that Nordic bees collected red clover pollen more frequently than did the Italian bees. Pedersen (95) found that the Italian bees increased on second crop and the Danish bees decreased and that the Italian bees were less often nectar thieves on both crops. Data published in a 277-page symposium on red clover pollination edited by Gubin (110) showed that Ukranian bees with slightly longer tongues than northern bees were slightly better pollinators and Caucasian bees with even longer tongues were nearly twice as valuable as the northern bees. Their findings were based on relative rates of increase in yields as one approached the apiary. Hunkeler (111) in Germany stated that Carniolan bees were ousting northern bees in red clover districts because they gathered more red clover honey and did a better job of pollination. In North America there has been little effort to evaluate or use one strain of bees over another for red clover pollination. Perhaps in areas where bumble-bees cut few holes in the corolla tubes, the question of honey bee races is of minor importance.

Training bees to red clover is another approach to increasing their nectar visits. Von Frisch (112) placed hives close to red clover fields and fed the bees in front of the hives with sugar water. The bees had to creep through a layer of fresh red clover flowers to reach the syrup. Afterwards they danced in the hive and other bees, perceiving the odor of the flowers on the dancers' bodies, searched for red clover. Von Frisch tried 12 paired experiments in different localities, and in every one visitation was higher to red clover fields adjacent to trained than to untrained colonies. Several Russian investigators tried similar experiments. As early as 1930 they simply steeped the florets in the feeding solution. In 1933 Gubin & Romashov (113), using extracted perfume, trained one group of colonies to red clover and another to willow herb and then reversed the training of both groups. Most Russian investigators now advocate this method of increasing honey bee nectar visits to red clover. Czech research workers, following up on the Russian experiments, sprayed hives and red clover fields with fennel (*Foeniculum officinale*) and aniseed (*Pimpinella anisum*) oils in sugar syrup [Cumakov (114)]. On the other hand, Minderhoud (115) in Holland and Valle (116) in Finland tried similar methods (using extracts as well as florets) without clear-cut results. Minderhoud claimed some success with spraying the crop with saccharose instead of plain sugar syrup. However, MacVicar and associates (117) in Canada sprayed honey on red clover without benefiting pollination.

Zivov & Skvorcov (118) surrounded plots of red clover with sowings of *Trifolium ambiguum* and harvested the latter when the red clover was in good bloom. They claimed excellent results with this method in commercial seed fields of four provinces in Russia. Apparently this procedure has not been tried in other countries or, if tried, was found unsuccessful and remained unreported.

Honey bees collect pollen as well as nectar from red clover. According to Skovgaard (119) the pollen collectors have two advantages over nectar

collectors: (*a*) they pollinate about twice as fast, and (*b*) there are no "inferior" pollen-gathering races. (This was said in defense of the northern races of bees, usually considered inferior in nectar collecting.) Schwan (109) in Sweden went a step further by showing a higher percentage of pollen collectors and more rapid pollen visits for the Nordic bees in comparison with Italian strains. Another obvious advantage of pollen collectors (not seen in the literature) is that they nearly always enter the flower from the top instead of using the side entrances made by bumble bees. The percentage of pollen gatherers on red clover is variable but usually quite substantial.

Skovgaard (119) stated that the percentage of pollen gatherers on red clover is associated with the pollen needs of the colonies involved. He did not state how the association was determined. In his studies pollen collectors averaged 30 per cent on early clover and 20 per cent on late clover. This difference may merely be an expression of the greater number of nectar collectors on the late clover. Butler (120) found an average of 40 per cent pollen collectors on red clover and concluded that visitation of both pollen and nectar collectors was determined by the height of the nectar in the corolla tubes. Woodrow (121) stated that most of the honey bees on red clover in Ohio collect pollen. He did not make a sharp distinction between pollen and nectar collectors, stating that the pollen collectors seem to receive the pollen unintentionally while probing for nectar. Dunham (122) stated that honey bees devote practically all their time on red clover bloom to collecting pollen.

Such discrepancies in observation probably result from variable complexes of competing bloom as well as variations in the nectar content of red clover. Wilsie & Johnson (123) concluded that red clover does not compete well with most other nectar-bearing plants for nectar-collecting honey bees but holds its own with all but the most attractive pollen plants for pollen collectors. MacVicar *et al.* (117) studied pollen-trap collections from an area where many sources of pollen were available and found red clover to be the dominant pollen in nearly every sample. Others have made similar findings [Braun *et al.* (124); Bohart (unpublished data)]. It is easy to see why the number of nectar collectors fluctuates widely from time to time and place to place, but the occasionally reported absence or near absence on red clover of honey bees of any kind [Plath (93); Valle (125)] is hard to understand. Perhaps the fields were very small and surrounded by strongly competing pollen sources.

Most observers have found a negative relationship between red clover seed yields and distance from colonies of honey bees. Wilsie (126) in a three-year study of two areas with fields extending as far as 16 miles from major bee yards, found significant decreases in seed yields at the greater distances. Walstrom *et al.* (127), working more intensively with smaller distances, found a distinct break in seed yields between 400 and 600 feet. MacVicar *et al.* (117) found a similar break between 500 and 800 yards away from the colonies. Braun *et al.* (124), found a break in both honey bee populations and

seed yields between 800 and 1200 feet and progressive declines up to 2000 feet. However, Harrison *et al.* (128) in Michigan found no significant differences in seed yields as the distance from the apiary was increased up to 1 mile.

Pollination by Wild Bees

All students of red clover have unanimously accorded bumble bees the highest place in red clover pollinating efficiency. Apparently the long-tongued species of bumble bees and red clover are "made for each other" [Brian (129)]. A number of observations on the working speed of bumble bees indicate that the queens pollinate about four times as many florets per minute as pollen-collecting honey bees and the workers from two to three times as many [Schwan (109)]. Pedersen (95) gave bumble bees 2.5 "bee units" in comparison with 1.0 for honey bees. With this as a basis he calculated that 0.5 bee unit per square meter should be able to pollinate enough red clover flowers to set a seed crop of 1000 to 1200 kg. per hectare.

The principal trouble with bumble bees as red clover pollinators seems to be their scarcity in most localities. Valle in Finland (116), Schwan in Sweden (109), Benoit & Gillard in Holland (130), and Bird in Quebec (131) have noted great population fluctuations from year to year, making seed production without honey bees very unreliable.

A shortcoming of bumble bees that applies principally to certain short-tongued species is their habit of cutting holes in the bases of the corollas to secure the nectar more easily [Brian (129)]. These holes are then used by other bees, and the flowers remain unpollinated. *Bombus terrestris* (Linnaeus) is the most common nectar thief in Scandinavia [Schwan (109); Pedersen (95)]. *Bombus terricola* Kirby and *B. occidentalis* Greene are well-known nectar thieves in North America. Unfortunately, when three species of bumble bees were introduced into New Zealand, *B. terrestris* was included [Cumber (132)].

Williams (133) in Wales and Morrison (134) in Ontario are among those reporting the importance of timing the bloom for seasonal peaks of bumble bee populations. In such northern latitudes they reported a small peak in June when the queens foraged and a much larger peak in late July when the workers appeared in large numbers. On the other hand, Pedersen (95) found twice as many bumble bees on the first crop as on the second. Apparently, timing the crop for bumble bee pollination must be done on the basis of local conditions.

Other pollinators are usually unimportant on red clover. However, as reported by Folsom (135) first-crop red clover may be pollinated in the Midwest to an important degree by species of *Tetralonia*, and the early second crop by species of *Melissodes*. Yamada & Ebara (136) reported *Eucera sociabilis* Smith as an important red clover pollinator in Japan, and Benoit & Gillard (130) give some credit to *Andrena wilkella* (Kirby) in Holland.

Many agronomists, entomologists, and naturalists of various kinds have

studied the biology of bumble bees and methods of domesticating them. It appears that no one has yet demonstrated any advantage to keeping bumble bees for pollination except for small-scale work in enclosures [Bohart & Pedersen (137)]. However, Hasselrot (138) in Sweden and Valle (139) in Finland have made efforts to develop bumble bee keeping for pollination on a field scale.

To summarize, nearly all authors are now in agreement on the following points concerning red clover pollination: (*a*) Red clover is self-sterile and requires pollination by bees. (*b*) Bumble bees, except for a few nectar-thieving species, are ideal pollinators although their populations are unpredictable and usually insufficient. (*c*) Honey bees are satisfactory pollinators providing that they are sufficiently concentrated in the area and competing pollen and nectar sources are kept at a minimum. (*d*) As a pollen source, but not as a nectar source, red clover competes well with other plants for the attention of honey bees. (*e*) Honey bee pollination is generally better on second-crop than first-crop bloom. (*f*) Nectar production and sugar concentration in red clover are greatest in warm, dry weather.

There is still disagreement as to the value of long-tongued honey bees, short-tubed flowers, scent training of nectar gatherers, and attracting bees with attractive flowers prior to red clover bloom.

The foregoing review should at least make it clear that a great deal of work is going on at the present time in the field of legume pollination. Experimental pollination studies are difficult and often costly because of the wide-ranging foraging habits of most pollinators and the large number of species involved. Some of the more complex problems such as the nature and significance of competition between pollinators will probably never be completely answered.

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